Antimicrobial disposition and interactions with gastrointestinal microflora in *Equidae*

A thesis submitted for the degree of

Doctor of Philosophy

by

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Animals are such agreeable friends - they ask no questions, they pass no criticisms

Mr. Gifil's Love Story George Eliot 1819-1880

The remedy is worse than the disease

Of seeming wise Francis Bacon 1561-1626

Contents

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Declaration

The contents of this thesis are the work of the author. The thesis has not been submitted previously to any university for the award of a degree. The following publications are based on the work contained in this thesis:

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Summary

Penicillin G was detected in plasma (>0.01 μg/ml) for up to 8 h in horses and 6 h in ponies and donkeys following intravenous administration at a dose rate of 10 mg/kg bwt. The elimination half-life (harmonic mean) of penicillin G was similar in horses, ponies and donkeys (38.95, 27.25 and 31.52 min, respectively). The short elimination half-life was reflected by a high mean CL_b of 514.46, 505.36 and 462.86 ml/h.kg, respectively. There was an increase in the mean number of coliforms (10^8-10^9/g) isolated from faeces following intravenous administration of penicillin G to ponies and donkeys. There was an increase in the mean number of *Clostridium spp.* (10^7/g) isolated from donkey faeces following intravenous administration of penicillin G. There were no marked alterations in the viable number of bacteria isolated from caecal liquor, or in caecal liquor pH following intravenous administration of penicillin G; however there was an increase in caecal liquor lactic acid concentrations to 59.5 mmol/l in 1/2 ponies only. Penicillin G was absorbed rapidly following administration by nasogastric tube to ponies at a dose rate of 10 mg/kg bwt, and peak concentrations of 0.09-0.19 μg/ml were reached at 0.5 h, but the systemic availability was low (0.12-0.34%). Peak concentrations of penicillin G in caecal liquor were high (4.96-157.12 μg/ml), and there were increases in the number of viable coliforms (10^7-10^{10}/ml), streptococci (10^7-10^{10}/ml) and *Clostridium spp.* (10^6-10^8/ml) isolated from caecal liquor. In addition there was an increase in caecal pH from 6.8-7.2 to 7.3-8.3. There were alterations in SCFA concentrations in caecal liquor following oral administration of penicillin G. Namely, an increase in lactic acid concentrations to 5.1-38.7 mmol/l, and a reduction in propionic acid concentrations to 0.0-3.7 mmol/l and butyric acid concentrations to 0.0-3.5 mmol/l, and a reduction in the proportions of propionic and butyric acids to 0.0% and 0.0-7.1%, respectively. The increase in lactic acid concentrations in caecal liquor was reflected by a slight increase in lactic acid concentrations in faeces to 6.5 mmol/kg. Incubation of penicillin G *in vitro* at 37 °C in an anaerobic environment for 3 and 24 h resulted in the destruction of 19.8 and 64.5%, respectively. Incubation of penicillin G at pH 1.9 for 1 h at room temperature resulted in the destruction of 84.7%. It appeared, from a single replicate, that around 90% of penicillin G was bound to hay at pH 1.9 and pH 7.0 *in vitro*.

Ampicillin was detected (>0.02 μg/ml) in plasma for 8 h in horses and 6 h in ponies and donkeys following intravenous administration at a dose rate of 10 mg/kg bwt. The elimination half-life (harmonic mean) was 60.88, 52.34 and 42.80 min in horses, ponies and donkeys, respectively. There was a statistically significant difference between the AUC and CLb in horses and donkeys. The mean AUCs were 44.17 μg.h/ml and 24.70 μg.h/ml and the CL_bs were 229.67 ml/h.kg and 418.80 ml/h.kg in horses and donkeys, respectively. There were no marked alterations in the number of viable bacteria isolated from

faeces following intravenous administration of ampicillin to horses, ponies and donkeys. There was an apparent increase in the concentrations of lactic acid in caecal liquor, up to a maximum of 44.4 mmol/l, following intravenous administration of ampicillin to ponies. Ampicillin was absorbed rapidly following administration by nasogastric tube to ponies at a dose rate of 10 mg/kg bwt. Maximum plasma concentrations of 0.74-2.30 μg/ml were measured at 0.5-0.75 h, but the systemic availability was low (1.20-5.54%). There were high concentrations (3.63-144.53 μg/ml) of ampicillin measured in caecal liquor following oral administration to ponies with cannulated caecal fistulas and consequently there were increases in the number of viable coliforms (10^7-10^{11}/ml), streptococci (10^8-10^{10}/ml), lactobacilli (10^9-10^{11}/ml) and *Clostridium spp.* (10^6-10^{10}/ml) isolated. The most marked trend in SCFA concentrations in caecal liquor following oral administration of ampicillin was an increase in lactic acid concentrations to a maximum of 51.6 mmol/l, a reduction in propionic acid concentrations to 0.0-3.8 mmol/l, and a reduction in the proportion of propionic acid to 0.0-3.8%. In addition, there was an increase in lactic acid concentrations in faeces to 35.6-64.2 mmol/kg. There were no marked alterations in the faecal dry matter content or consistency. Approximately 64% of ampicillin was inactivated by anaerobic incubation in caecal liquor for 24 h at 37 °C. Little or no ampicillin was destroyed by incubation at pH 1.9 for 1 h at room temperature. Incubation of ampicillin with hay at 37 °C for 3 h resulted in inactivation of around 59% at pH 7.0.

Amikacin was measured (>0.02 μg/ml) in plasma for 12 h in horses and donkeys and 8 h in ponies following intravenous administration at a dose rate of 6 mg/kg bwt. The elimination half-life (harmonic mean) of amikacin was similar in horses, ponies and donkeys (2.84, 1.60 and 1.93 h, respectively), and the CLb (mean) was quite slow (45.19, 82.37 and 57.98 ml/h.kg, respectively). There were no marked alterations in the number of viable bacteria isolated, caecal liquor pH or caecal liquor and faecal SCFA concentrations. Amikacin was not detected (<0.02 μg/ml) in plasma following administration by nasogastric tube to ponies with cannulated caecal fistulas, however there were high concentrations of amikacin measured in caecal liquor (maximum 16.19-99.37 μg/ml). Despite the high concentrations of amikacin in caecal liquor, there were no marked alterations in the number of viable bacteria isolated from caecal liquor, however there was a reduction in caecal liquor pH to <6.6, and there was an increase in caecal liquor lactic acid concentrations to a maximum of 24.4 mmol/l. The increase in caecal liquor lactic acid concentrations were reflected by a maximum lactic acid concentration of 15.4 mmol/kg in faeces. There were no marked alterations in the faecal dry matter content or consistency. Incubation of amikacin in caecal liquor *in vitro* resulted in little loss in activity; 90.8% and 85.0% remained following 3 and 24 h incubation, respectively. There was little loss of activity (14.4%) of amikacin following incubation *in vitro* at pH 1.9 for 1 h at room temperature. There appeared to be a

high percentage of activity of amikacin lost (around 95%) following incubation *in vitro* with hay at 37 °C for 3 h at pH 1.9 and pH 7.0.

Oxytetracycline was detected (>0.08 μg/ml) in plasma for 96 h in horses, 72 h in ponies and 48 h in donkeys following intravenous administration at a dose rate of 10 mg/kg bwt. The elimination half-life (harmonic mean) of oxytetracycline was 11.69, 11.77 and 5.40 h. There were statistically significant ($P<0.05$) differences in AUC, AUMC and CLb between horses, ponies and donkeys following intravenous administration at a dose rate of 10 mg/kg bwt. The mean AUC and AUMC in horses (253.53 μg.h/ml and 3949.47 $\mu g.h^2/ml$) were larger than in donkeys (109.45 μg.h/ml and 780.41 $\mu g.h^2/ml$), and the mean CLb in horses (39.31 ml/h.kg) was lower than in donkeys (91.37 ml/h.kg). There were increases in the mean number of viable streptococci ($10^9/g$), lactobacilli (10^9-$10^{11}/g$) and *Clostridium spp.* ($10^7/g$) isolated from faeces following intravenous administration of oxytetracycline to horses, and there was an increase in the mean number of coliforms ($10^8/g$) isolated from donkey faeces. There were increases in the mean lactic acid concentrations in faeces to 25.1 and 6.4 mmol/kg in horses and ponies, respectively and there was a reduction in the mean dry matter content of faeces to <17% in all three groups. There was a maximum concentration of oxytetracycline of 2.41 μg/ml in caecal liquor following intravenous administration to ponies with cannulated caecal fistulas at a dose rate of 10 mg/kg bwt which was usually less than 17% of the administered dose. There were no marked alterations in the number of viable bacteria isolated from caecal liquor following intravenous administration of oxytetracycline to ponies with cannulated caecal fistulas although there were increases in caecal liquor lactic acid concentrations to a maximum of 40.2 mmol/l, and faecal lactic acid concentrations to a maximum of 25.7 mmol/kg. The ponies were depressed and anorectic following oral administration of oxytetracycline at a dose rate of 10 mg/kg bwt. Oxytetracycline was absorbed quite rapidly, but the systemic availability was low (0.43-1.04%). Peak plasma concentrations of 0.38-0.84 μg/ml were measured at 0.5-1.5 h after administration, and there were high concentrations of oxytetracycline measured in caecal liquor (18.40-80.50 μg/ml). *Salmonella typhimurium* phage type 204c, which was susceptible *in vitro* to furazolidone, was isolated from one pony on two separate occasions following oral administration of oxytetracycline. There were increases in the number of viable coliforms (10^7-10^{11}/ml), streptococci (10^8-10^{11}/ml), lactobacilli (10^8-10^{10}/ml) and *Clostridium spp.* (10^6-10^7/ml) isolated from caecal liquor following oral administration of oxytetracycline. There was an increase in lactic acid concentrations up to a maximum of 53.9 mmol/l, which was reflected by an increase in lactic acid concentrations in faeces up to a maximum of 65.4 mmol/kg. There were no marked alterations in the faecal dry matter content although the faecal consistency was reported as soft following oral administration of oxytetracycline. There was little alteration (17% reduction) in oxytetracycline activity

following anaerobic incubation of oxytetracycline in caecal liquor at 37 °C for 3 and 24 h and following incubation of oxytetracycline at pH 1.9 for 1 h at room temperature (4% reduction). There was an increase in the number of coliforms (10^{10}/ml) and *Clostridium spp.* (10^{6}/ml) isolated from caecal liquor following *in vitro* incubation with 80 μg/ml oxytetracycline for 24 h.

Abbreviations

A	acetic acid
ALT	alanine transaminase
AST	aspartate transaminase
ATCC	American Type Culture Collection
aufs	absorbance units full scale
B	butyric acid
B.	*Bacteroides*
Bas	basophils
bwt	bodyweight
C.	*Clostridium*
cfu	colony forming units
cm	centimetres
D-	*dextro*-rotatory
dl	decilitres
E.	*Escherichia*
e. g.	for example
Eos	eosinophils
et al.	and others
F	systemic availability
fl	femtolitres
FW	formula weight
g	grams
g	gravity, 10^{-11} N.m/s^2
GGT	gamma glutamyl transferase
GLC	gas-liquid chromatography
h	hours
Hb	haemoglobin concentration

Hct	haematocrit
Hg	mercury
HPLC	high performance liquid chromatography
i. e.	that is
IB	isobutyric acid
id	internal diameter
iu	international units
IV	isovaleric acid
kg	kilogram
L	lactic acid
l	litre
λ	wavelength
L-	*laevo*-rotatory
L.	*Lactobacillus*
lb	pound (weight)
$\log_{10}$	logarithm base 10
Lym	lymphocytes
M	molar
m	metres
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean cell volume
mg	milligram
MIC	minimum inhibitory concentration
min	minutes
ml	millilitre
mM	millimolar
mm	millimetres

mmol	millimoles
Mon	monocytes
mV	millivolts
Neu	neutrophils
nm	nanometres
No.	number
NS	no sample
NVFA	non-volatile fatty acids
obs	observed values
p	probability
P	propionic acid
P+B	propionic plus butyric acids
PCT	plateletcrit
PDW	platelet distribution width
pg	picograms
pH	negative logarithm of hydrogen ion concentration
ppm	parts per million
r	Pearson linear correlation coefficient
RCC	red cell count
s	seconds
S.	*Salmonella*
SAP	alkaline phosphatase
SCFA	short-chain fatty acids
SD	standard deviation
SEM	standard error of mean
spp.	*bacterial species*
TSI	triple sugar iron
U	units
uv	ultraviolet

V	valeric acid
VFA	volatile fatty acid
w/v	weight/volume
WCC	white cell count
µg	micrograms
µl	microlitres
µm	micrometres
µmol	micromoles
°C	degrees Centigrade

Pharmacokinetic terms

A1, A2 Zero-time plasma drug concentration intercepts (μg/ml) of a bi-phasic disposition curve where A1 is based on the terminal exponential slope.

A3 Zero-time plasma concentration (μg/ml) intercept of initial exponent of a tri-phasic disposition curve.

AUC Total area under the drug concentration versus time or zero moment curve (μg.h/ml) from time 0 to time ∞ after administration of a single dose calculated using the trapezoidal rule (obs) and from $^{A3}/_{B3} + {}^{A2}/_{B2} + {}^{A1}/_{B1}$.

AUMC Total area under the drug concentration time versus time or first moment curve (μg.h^2/ml) from time 0 to time ∞ after administration of a single dose calculated using the trapezoidal rule (obs) and from $^{A3}/_{B3^2} + {}^{A2}/_{B2^2} + {}^{A1}/_{B1^2}$.

B1, B2 Hybrid rate constants (exponents) (/h) of a bi-phasic disposition plasma concentration versus time curve related to the slopes of the distribution and elimination phases of a bi-exponential drug disposition curve, where B2 is the overall elimination rate constant.

B3 Hybrid rate constant (/h) of the initial exponent of a tri-exponential disposition curve.

CL_b Body clearance (ml/h.kg) of a drug which represents the sum of all clearance processes in the body calculated from $^{dose}/_{AUC}$.

C_{max} Maximum plasma concentration (μg/ml) following oral drug administration.

Cp0 Initial concentration of a drug in plasma (μg/ml) following administration of an intravenous bolus administration calculated from the sum of the coefficients.

F Systemic availability calculated as the fraction of the dose that enters the systemic circulation intact following oral administration by dividing AUC_{obs} following oral administration by AUC_{obs} following intravenous administration and expressing it as a percentage.

k_{12}, k_{21}, k_{13}, k_{31} First-order transfer rate constants calculated for drug distribution between the central and peripheral compartments of a multi-compartment model.

k_{el} First-order elimination rate constant (/h) for the disappearance of a drug from the apparent central compartment calculated from the ratio of body clearance to the apparent volume of the central compartment.

MAT mean absorption time (h) calculated from the difference between the MRT following a single oral administration and MRT following a single intravenous administration.

MRT Mean residence time (h), quantitative estimate of the persistence of a drug in the body, or the time for 63.2% of a drug to be eliminated. Calculated from $AUMC_{obs}/AUC_{obs}$.

$t^1/_2$ B1 Elimination (biological) half-life (h or min) following intravenous administration or time taken for 50% of a drug to be eliminated, or apparent half-life following oral administration, calculated from $0.693/B1$.

$t^1/_2$ B2 and B3 Distribution half-lives (h or min) following intravenous administration or apparent half-life (B2) following oral administration, calculated from $0.693/B2$ and $0.693/B3$.

V_c Apparent volume of the central compartment (ml/kg) calculated from $dose/Cp0$.

Vd_{area} Apparent volume of distribution (ml/kg) based on the AUC, proportionality constant relating the plasma drug concentration to the amount of drug in the body calculated from $dose/AUC.B1$.

Vd_{ss} Apparent volume of distribution (ml/kg) at steady state concentration calculated from $dose_{(intravenous)}.AUMC/AUC^2$.

1 Introduction

1.1 Antimicrobial agents

An antibiotic is defined as a substance which kills or inhibits the growth of microorganisms. The original antibiotics were produced as the by-products of fermentation of microorganisms, however some antibiotic compounds are synthesized and this has led to the use of the more general term of antimicrobial agent to describe substances which, at low concentrations, antagonize the growth of microorganisms.

Antimicrobial agents can be allocated to different groups according to a variety of factors. The classification of antimicrobial agents into those which result in cell death *in vitro*, bactericidal antimicrobial agents, and those which cause stasis of bacterial growth *in vitro* and those which rely on competent host defence mechanisms *in vivo*, bacteriostatic antimicrobial agents, is ambiguous. Classification as bactericidal and bacteriostatic requires specific organisms and growth conditions to be defined, and therefore is inapplicable to a clinical situation. Classification of antimicrobial agents according to their mechanism of action is less confusing. Antimicrobial agents can be allocated to groups such as compounds which inhibit cell wall synthesis (bacitracin, penicillins, cephalosporins, isoniazid, vancomycin), inhibit plasma membrane function (polymyxins), irreversibly inhibit protein synthesis (aminoglycosides, aminocyclitols), reversibly inhibit protein synthesis (chloramphenicol, lincosamides, macrolides, tetracyclines), inhibit nucleic acid synthesis (metronidazole, nitrofurans, rifamycins), inhibit intermediary metabolism (trimethoprim, sulphonamides, ionophores), and interfere with deoxyribonucleic acid packaging and transcription (quinolones).

The spectrum of activity of an antimicrobial agent can be described as either narrow or broad. An antimicrobial agent with a narrow spectrum of activity affects a relatively narrow range of organisms. A broad spectrum antimicrobial agent affects a wide variety of Gram positive and Gram negative bacteria, and may affect other organisms, such as rickettsias. Penicillin G affects Gram positive and a limited range of Gram negative (*e. g. Pasteurella spp.*) bacteria. Aminoglycosides affect aerobic Gram negative bacteria, in the main, and are of no value in an anaerobic environment. Penicillins with an extended spectrum of activity, such as ampicillin and amoxicillin, are active against Gram positive bacteria and some Gram negative bacteria but are not active against bacteria that produce ß-lactamase enzymes. The tetracyclines affect many Gram positive and Gram negative bacteria, and rickettsia, mycoplasma and ehrlichia.

1.2 Antimicrobial therapy

Antimicrobial agents have been used commonly for veterinary therapy, prophylaxis and as low level feed additives for growth promotion (Powers and Mercer, 1980). A number of factors should be considered prior to the selection of an antimicrobial agent (Brumbaugh, 1987, Baggot and Prescott, 1987, Sandford, 1976, Spurlock and Hanie, 1989). Firstly, the organism must be susceptible to the action of the antimicrobial agent. Secondly, an appropriate drug concentration must be present at the site of infection for sufficient time and the drug must retain its activity at the site of infection. Finally, safety and cost should be taken into consideration.

Information on the susceptibility of bacterial pathogens to different antimicrobial agents is available in the literature (Hirsh and Jang, 1987). Bacterial isolation and antimicrobial susceptibility testing of bacterial isolates from individual cases allows therapy to be tailored to suit the individual. There are a number of different tests used to quantify the susceptibility of bacterial isolates to antimicrobial agents, such as minimum inhibitory concentrations. This type of test provides useful information on the appropriate concentrations of an antimicrobial agent required to affect susceptible bacteria. However, care should be taken in the interpretation of the results of this type of *in vitro* diagnostic technique, since it is carried out under standardized, controlled laboratory conditions, including the use of specific growth media and optimal conditions for bacterial growth, and may not reflect the clinical situation (Brumbaugh, 1987, Prescott and Baggot, 1985).

Pharmacokinetics is a mathematical description of drug disposition, usually in plasma, with time in the body of an animal. There are a number of computer software programmes available for the analysis of plasma concentration versus time data. Studies of plasma disposition of a drug, usually following a single intravenous, intramuscular or oral administration, are used to calculate the optimal frequency of drug administration required to attain and maintain desired therapeutic plasma concentrations (English and Roberts, 1979). In addition, studies that examine drug excretion (*e. g.* renal or biliary) and tissue concentrations provide additional useful information on the absorption, distribution and clearance of a drug. The majority of pharmacokinetic data is collected in normal animals and there is little information on the effects of clinical or subclinical disease on drug disposition.

1.3 Adverse reactions to antimicrobial agents

An adverse reaction is defined as any response to a drug, excluding therapeutic failures, which is noxious and unintended and which occurs following administration, for prophylaxis, diagnosis or therapy, of an appropriate dose of a drug and within a reasonable

time frame of administration (Baggot, 1989, Davis, 1987, Ruckebusch, 1983). These effects can be direct or indirect, and include acute immune-mediated reactions (*e. g.* hypersensitivity), acute non-immune mediated drug reactions (*e. g.* cardiovascular and haematologic reactions, drug fever), and toxic reactions (*e. g.* nephrotoxicity, hepatotoxicity) (Baggot, 1989, Davis, 1987, English and Roberts, 1983, Powers and Mercer, 1980, Ruckebusch, 1983). Toxic drug reactions are, by definition, drug-induced reactions that result in damage to an organ due to an alteration in its structure or function. Biological or metabolic effects, such as alterations in the gastrointestinal microflora, should be included in this category. Although, the complex mechanisms of some toxic drug reactions are recognized (*e. g.* the nephrotoxicity of the aminoglycoside antimicrobial agents) many mechanisms of drug toxicity remain obscure (Davis, 1987). In addition, interpretation of an adverse drug reaction is made more complex by the presence of more than one drug, and by reaction to components of the dosage form other than the active agent.

The adverse effects of antimicrobial agents on the alimentary tract have been well recognized as a complication of modern antimicrobial therapy in several species (Mackellar *et al.*, 1973, Owen, 1975, Ricketts and Hopes, 1984, Roberts and English, 1979, White and Prior, 1982). Gastrointestinal side effects can range from mild diarrhoea to severe and potentially life-threatening antimicrobial-associated colitis (Baggot, 1989, Butzler, 1982, Cheymol *et al.*, 1986, Ewe, 1988).

The development of antimicrobial-associated colitis due to *C. difficile* infection may be due to inappropriate antimicrobial therapy. In one survey, only 25% of patients who developed *C. difficile* toxin positive stool had been treated with a suitable antimicrobial agent for their primary infection, and a further 25% had an appropriate diagnosis but were treated incorrectly (Yarinsky and Wheeler, 1990). In addition, fatal pseudomembranous colitis has been related to the dosage and duration of antimicrobial therapy (Ramirez-Ronda and Sandford, 1975). Hence the importance of isolating bacterial pathogens, testing the susceptibility of bacterial isolates to antimicrobial agents, and selecting and administering an antimicrobial agent.

Any antimicrobial agent which remains in the gastrointestinal lumen, following oral administration, or which is excreted in bile or through the intestinal mucosa, following parenteral or oral administration, may alter or destroy the normal gastrointestinal microflora, and allow overgrowth of non-susceptible or pathogenic microorganisms and the development of diarrhoea (Bell *et al.*, 1978, Butzler, 1982, Jusko, 1975, Larson *et al.*, 1978, Levine and Lamont, 1982, Mittermayer, 1989, Nord, 1991, Powers and Mercer, 1980, Swerczek, 1979). Alteration in the commensal microflora may result in the exacerbation of existing disease or the development of a secondary infection, thus the value

of antimicrobial agents in the treatment of intestinal disease is questionable (Powers and Mercer, 1980, Swerczek, 1979). The development of ulcerative colitis, as a sequel to dysbiosis of the colonic bacteria, is due to bacterial enteroadhesion, the release of bacterial peptides and the formation of bacterial secondary metabolites (Roediger, 1980).

Adverse reactions to antimicrobial agents have been reported in the horse but either occur infrequently or are not observed or reported (English and Roberts, 1983). In France, around 0.8% of antimicrobial treatments in horses are reported as adverse reactions (Puyt, 1989).

The horse may be particularly susceptible to adverse reactions to antimicrobial agents due to its digestive physiology and to the limited number of antimicrobial agents indicated specifically for use in this species (Puyt, 1989). This may result in the use of inappropriate antimicrobial agents in the equine. Surprisingly, gastrointestinal adverse reactions have received scant attention despite the value of individual horses and the problems of litigation (Roberts and English, 1979).

Antimicrobial agents which undergo enterohepatic circulation, *e. g.* ampicillin and oxytetracycline, may affect the microorganisms of the caecum and colon following parenteral or oral administration, and this may limit their clinical usefulness in adult horses (Baggot and Prescott, 1987, Cook, 1973, Owen, 1975, Roberts and English, 1979). Oral administration of antimicrobial agents has been uncommon in the horse due to the low systemic availability of antimicrobial agents. Systemic availability may be poor even when the physicochemical properties of an antimicrobial agent are suitable for absorption due to poor dissolution performance, hepatic or bacterial metabolism, or drug instability (*e. g.* penicillin G and erythromycin) (Baggot, 1984). Following oral administration, any antimicrobial agent remaining in the gastrointestinal lumen may alter the commensal microflora and produce changes in SCFA concentrations, and unabsorbed SCFA may precipitate or exacerbate osmotic diarrhoea (Baggot, 1984, Clark and Becht, 1987, Ensink *et al.*, 1992, Owen, 1975, Roberts and English, 1979). The hazard of inducing dysbacteriosis is smallest when the concentration of active antimicrobial agent reaching the caecum and colon is low (Ensink *et al.*, 1992). This occurs following the complete absorption of an antimicrobial agent in the jejunum, in the absence of biliary elimination, or following the administration of an inactive prodrug.

1.4 Pseudomembranous colitis

In man, the most severe gastrointestinal adverse reaction to antimicrobial agents is pseudomembranous colitis which was first documented in 1893 which preceded the discovery of antimicrobial agents. Pseudomembranous colitis is a reasonably common side effect of antimicrobial administration caused by overgrowth of the toxin producing, spore

forming, Gram positive, anaerobic rod, *C. difficile* (Meyer-Kawohl and Bockemuhl, 1986, O'Connor, 1981, Trnka and Lamont, 1984). In fact, *C. difficile* is one of the commonest pathogens of the lower intestinal tract with infection almost always occurring during or after antimicrobial treatment (Guandalini *et al.*, 1988, Kager *et al.*, 1979, Meyer-Kawohl and Bockemuhl, 1986, Trnka and Lamont, 1984). Bacteria other than *C. difficile* have been involved in the development of pseudomembranous colitis. Aronsson *et al.* (1981) isolated 5 strains of *Clostridium spp.* that were not *C. difficile*, and Borriello *et al.* (1984) and Machida *et al.* (1989) isolated *C. perfringens.* In addition, colitis may be due to infection with *Citrobacter spp.* (Barbulescu, 1972), *Campylobacter jejuni* (Butzler, 1982), toxin-producing *E. coli* (O157:H7) (Griffin *et al.*, 1990) or staphylococci (including methicillin-resistant staphylococci) (Chovancova and Lucansky, 1970, Chubachi *et al.*, 1989, Hori *et al.*, 1989, Klimek *et al.*, 1976).

Clostridium difficile does not colonize the normal healthy adult gastrointestinal tract. Antimicrobial therapy alters the normal gastrointestinal microflora and allows *C. difficile* colonization to take place (Borriello *et al.*, 1987, Meyer-Kawohl and Bockemuhl,1986). In the normal gastrointestinal lumen, some component of the commensal microflora may prevent *C. difficile* colonization (Aronsson *et al.*, 1982, Trnka and Lamont, 1984).

Clostridium difficile produces two toxins (toxin A or enterotoxin and toxin B or cytotoxin) (Aronsson *et al.*, 1982, Lima *et al.*, 1988, Lima *et al.*, 1989, Tucker *et al.*, 1990). *Clostridium difficile* toxin produces cytotoxic damage to the colonic mucosa, which can lead to the development of diarrhoea (Mittermayer, 1989, Trnka and Lamont, 1984). One test of the cytotoxic effect of the these toxins is the oral inoculation of hamsters with an isolate of *C. difficile*. High virulence of *C. difficile* isolates has been defined as the ability of the organism to colonize the gastrointestinal lumen in the hamster, to produce disease, and to generate high concentrations of enterotoxin rapidly (Borriello *et al.*, 1987). There is wide variation in the virulence of different isolates of *C. difficile*. Strains of *C. difficile* isolated from domestic pets may be of low virulence for the hamster; although *C. difficile* is present after oral inoculation, caecal concentrations of enterotoxin and cytotoxin are low (Borriello *et al.*, 1987).

Clostridium difficile is an infectious organism and human cases of pseudomembranous colitis have occurred in groups or clusters (Cannon *et al.*, 1988). The major mode of spread of *C. difficile* infection is *via* the environment (Delmee *et al.*, 1988, Kaatz *et al.*, 1988, Kim *et al.*, 1981, Levine and Lamont, 1982, Meyer-Kawohl and Bockemuhl, 1986), but infection may occur after contact with an infected individual (Cerquetti *et al.*, 1989, Kim et al., 1981, Meyer-Kawohl and Bockemuhl, 1986, Möllby *et al.*, 1985, Wilson *et al.*, 1982, Wust *et al.*, 1982).

In Sweden (1980-1982), *C. difficile* or toxin was present in 2% of the general population, in 3% of diarrhoea cases unrelated to antimicrobial administration, and in 18% of patients with antimicrobial-associated diarrhoea (Möllby *et al.*, 1985). However, *C. difficile* colitis was recorded as an infrequent occurrence in adults in Peru (Sanchez *et al.*, 1990). Although, *C. difficile* appears to be an unusual infection in adults it has been reported as being endemic in longterm care facilities, particularly where there are geriatric patients (Rybolt *et al.*, 1989). Pseudomembranous colitis usually occurs in hospitalized patients. However, it has been reported in outpatients, where the number of unreported cases may be very high, since only severe cases of diarrhoea are investigated thoroughly (Jaeger *et al.*, 1981, Mittermayer, 1989).

The demonstration of *C. difficile* toxin is not associated exclusively with antimicrobial administration or the development of diarrhoea. In one study, *Clostridium difficile* toxin positive stool samples were recorded from 33% and cytotoxin from 50% of patients without diarrhoea, and similar results were obtained from patients with diarrhoea (Surawicz *et al.*, 1989). However, *C. difficile* was cultured, or toxin demonstrated, from stool samples of 90% of patients with pseudomembranous colitis, but neither were demonstrated in healthy human adults or in other intestinal diseases, except for cases of inflammatory colitis (Saco *et al.*, 1981). Thomas *et al.* (1990) reported that *C. difficile* infection was responsible for 20-25% of cases of antimicrobial-associated diarrhoea. Similarly, Trnka and Lamont (1984) noted that *C. difficile* toxin was demonstrable in stool filtrates of 33-50% of mild cases and nearly all severe cases of pseudomembranous colitis but Mogg *et al.* (1982) reported that only 44.7% (17/38) of patients with severe antimicrobial-associated post-operative diarrhoea had demonstrable faecal *C. difficile* or toxin.

The incidence of pseudomembranous colitis has ranged from 0.2% (Cheymol *et al.*, 1986) and 0.75% (Cannon *et al.*, 1988) to 1% (Cheymol *et al.*, 1986) of hospital patients treated with antimicrobial agents, 0.55% of surgical cases (Barile *et al.*, 1978) and 4% of patients that have undergone orthopaedic surgery (Cannon *et al.*, 1988). Colonic complications, including pseudomembranous colitis, occur in 1.9% of renal transplant cases (Flanigan *et al.*, 1988). Complications occurred in 5-10% of cases following colorectal surgery, due to a failure to prevent post-operative infections by antimicrobial prophylaxis (Alexander and Becker, 1988). It has been suggested that the incidence of post-operative colitis is increasing (Barzilai *et al.*, 1985, Hori *et al.*, 1989).

1.5 Colitis and *C. difficile* infection in human neonates

An abnormal flora may result if there is a delay in the normal bacterial colonization of the gastrointestinal tract. An abnormal gastrointestinal microflora may occur as a result of a

number of factors such as antimicrobial treatment (Lawrence *et al.*, 1982). In the human neonate, necrotizing colitis is thought to be caused by toxin production by an abnormal gastrointestinal microflora (George *et al.*, 1979, Lawrence *et al.*, 1982).

Commonly, neonates are asymptomatic carriers of *C. difficile* infection (Viscidi *et al.*, 1981). Colonization of the gastrointestinal tract of human neonates with *C. difficile* has ranged from 2-52% (Wilson *et al.*, 1982), and 25-60% of healthy human infants have been shown to carry *C. difficile* as part of their normal gastrointestinal microflora (Meyer-Kawohl and Bockemuhl, 1986, Mittermayer, 1989, Zweiner *et al.*, 1989). Thus, the isolation of the *C. difficile* or the demonstration of toxin cannot be linked exclusively to clinical signs of colitis in children (Meyer-Kawohl and Bockemuhl, 1986, Mittermayer, 1989). Moreover, *C. difficile* infection in children is not associated exclusively with antimicrobial therapy. Children with inflammatory bowel disease (*e. g.* Crohn's disease) can develop toxin negative *C. difficile* infection in the absence of exposure to antimicrobial agents (Hyams and McLaughlin, 1985). In one study, *C. difficile* was isolated from and toxin was detected in 28.9% (13/45) and 26.7% (12/45), respectively, of human neonates not exposed to antimicrobial agents but only 8.7% (2/23) and 4.4% (1/23), respectively, of older children (Viscidi *et al.*, 1981).

1.6 Antimicrobial agents associated with colitis in man

In humans, administration of nearly all antimicrobial preparations has triggered the development of *C. difficile* colitis (Ewe, 1988, Hecht and Olinger, 1989, Ledger and Puttler, 1975, Mittermayer, 1989). The risk of developing *C. difficile* colitis is increased when broad spectrum antimicrobial preparations are administered (Colatutto *et al.*, 1989, McKinley *et al.*, 1982, Mittermayer, 1989, Slagle and Boggs, 1976). Antimicrobial-associated colitis is associated more commonly with therapy using a combination of antimicrobial agents. Pseudomembranous colitis has been recorded as a complication of multiple antimicrobial therapy (Chua and Jackson, 1979, Church and Fazio, 1986). In one retrospective study, only 32.3% of patients with demonstrable *C. difficile* infection had been treated with a single antimicrobial agent (Church and Fazio, 1986).

Treatment with narrow or broad spectrum antimicrobial agents that inhibit bacterial cell wall synthesis has resulted in the development of colitis. Mild gastrointestinal side effects, such as nausea, diarrhoea or vomiting, occurred in 3.6-6.8% of patients following oral treatment with ß-lactam antimicrobial agents (Buchi and Casey, 1988, Newman *et al.*, 1985). Pseudomembranous colitis has been recorded following the administration of the penicillins (Aronsson *et al.*, 1982, Cheymol *et al.*, 1986, Chua and Jackson, 1979, Leung *et al.*, 1985). This has included both narrow spectrum penicillins, such as penicillin G (Borriello *et al.*, 1984, Buts *et al.*, 1977, McKinley *et al.*, 1982, Zweiner *et al.*, 1989), penicillin V

(Drapkin *et al.*, 1985), and flucloxacillin (Borriello *et al.*, 1984, Cannon *et al.*, 1988), and extended spectrum, semisynthetic penicillins, such as ampicillin (Buts *et al.*, 1977, Jaeger *et al.*, 1981, Jusko, 1975, Keshavarzian *et al.*, 1984, Leung *et al.*, 1985, McKinley *et al.*, 1982, Zweiner *et al.*, 1989) and amoxicillin (Zweiner *et al.*, 1989). In addition, treatment with the cephalosporins has resulted in the development of pseudomembranous colitis (Church and Fazio, 1986, Drapkin *et al.*, 1985, Hori *et al.*, 1989, Leung *et al.*, 1985, Marsh, 1984, McKinley *et al.*, 1982, Pierce *et al.*, 1982, Surawicz *et al.*, 1989, Zweiner *et al.*, 1989). This has included a variety of different first generation cephalosporins, such as cefazolin (Clarke *et al.*, 1990a), cephradine (Cannon *et al.*, 1988) and cefuroxime (Adams *et al.*, 1985, Borriello *et al.*, 1984, Cannon *et al.*, 1988, Morris *et al.*, 1984). Treatment with the cephalosporins, in particular the third generation cephalosporins (*e.g.* cefotaxine, moxalactam), is around 37 times more likely to result in the development of *C. difficile* colitis than penicillin V, and patients treated with the ß-lactamase resistant penicillins (*e. g.* methicillin, nafcillin, cloxacillin, flucloxacillin) proved to be at an even greater risk (Aronsson *et al.*, 1982). Intravenous administration of vancomycin resulted in the development of *C. difficile* colitis in a patient undergoing chronic haemodialysis, possibly due to the prolonged elevation of serum concentrations of vancomycin in patients with renal failure (Hecht and Olinger, 1989).

The development of *C. difficile* colitis has been associated with the administration of antimicrobial agents that inhibit bacterial protein synthesis. Pseudomembranous colitis has been recorded following administration of the aminoglycosides (Ewe, 1988, Leung *et al.*, 1985, Pierce *et al.*, 1982), including streptomycin (Chua and Jackson, 1979) and gentamicin (Borriello *et al.*, 1984, Boyd and Den Besten, 1976, Clarke *et al.*, 1990a, Leung *et al.*, 1985). The development of pseudomembranous colitis has been reported following administration of the macrolides spiramycin (Di Febo *et al.*, 1982) and erythromycin (McKinley *et al.*, 1982). The passage of watery stools, with or without an increased frequency of bowel action, occurs commonly in association with clindamycin therapy (Spiller *et al.*, 1984) and antimicrobial-associated colitis has been reported following administration of lincomycin or clindamycin (Boyd and Den Besten, 1976, Burdon *et al.*, 1981, Buts *et al.*, 1977, Cheymol *et al.*, 1986, Clarke *et al.*, 1990a, Dhawan and Thadepalli, 1982, Ewe, 1988, McKinley *et al.*, 1982, Pierce *et al.*, 1982, Ramirez-Ronda and Sandford, 1975, Surawicz *et al.*, 1989, Zweiner *et al.*, 1989). The risk of developing *C. difficile* colitis after lincosamide treatment was reported to be around 63 times greater than after treatment with penicillin V (Aronsson *et al.*, 1982), moreover colitis was 3-4 times more likely to develop following oral, compared with parenteral, lincosamide therapy (Dhawan and Thadepalli, 1982). The tetracyclines, including tetracycline (Boyd and Den Besten, 1976) and doxycycline (Chua and Jackson, 1979), have been reported to result in the development of pseudomembranous colitis.

Leung *et al.* (1985) reported the development of colitis following treatment with metronidazole, an inhibitor of bacterial nucleic acid synthesis.

Antimicrobial agents which inhibit bacterial intermediate metabolism have been associated with the development of colitis. Pseudomembranous colitis has been reported to develop following administration of the sulphonamides (Ewe, 1988) and the potentiated sulphonamides (trimethoprim/sulphonamide combinations) (Borriello *et al.*, 1984, Ewe, 1988, McKinley *et al.*, 1982, Surawicz *et al.*, 1989).

In patients with antimicrobial-associated colitis, the duration of diarrhoea and the presence of rectal pseudomembranes have not been associated with the administration of a specific antimicrobial agent (Burdon *et al.*, 1981). Moreover, it has been impossible to correlate the antimicrobial agents involved in the development of pseudomembranous colitis with increased resistance of *C. difficile* to antimicrobial agents (Aronsson *et al.*, 1981). Clinical isolates of *C. difficile* did not show any increased resistance to the penicillins or clindamycin (Aronsson *et al.*, 1982). This suggests that factors other than antimicrobial susceptibility, such as antimicrobial concentrations in the gastrointestinal tract or alterations in the normal gastrointestinal microflora, may play a role in the development of colitis.

1.7 Other factors associated with colitis in man

Antimicrobial administration may result in the development of colitis but there are a number of other important factors involved. Firstly, impaired host resistance due to severe underlying disease, *e. g.* acute promyelocytic leukaemia, is common in patients that develop colitis following antimicrobial therapy (Borriello *et al.*, 1984, Chubachi *et al.*, 1989, Dhawan and Thadepalli, 1982, Leung *et al.*, 1985, Meyer-Kawohl and Bockemuhl, 1986, Nord, 1991). Similarly, ovarian cancer chemotherapy (Satin *et al.*, 1989), and treatment involving enemas (Pierce *et al.*, 1982), nasogastric intubation (Pierce *et al.*, 1982, Surawicz *et al.*, 1989) or surgery (Clarke *et al.*, 1990a, Hori *et al.*, 1989, Ledger and Puttler, 1975, McKinley *et al.*, 1982, Meyer-Kawohl and Bockemuhl, 1986, Pesce *et al.*, 1984, Pierce *et al.*, 1982) have been associated with the development of antimicrobial-associated colitis. The length of the surgery and inadequate antimicrobial prophylaxis in the peri-operative period predispose patients to a high rate of post-operative infections (Alexander and Becker, 1988).

There is circumstantial evidence to support the role of faecal stasis in the pathogenesis of *C. difficile* related disease (Aubia *et al.*, 1981, Church and Fazio, 1986, Levine and Lamont, 1982, Percy and Christensen, 1985, Zweiner *et al.*, 1989). Many patients who developed pseudomembranous colitis were receiving treatment with medicines which produce faecal stasis (Church and Fazio, 1986) or had an underlying disease associated with faecal stasis,

e. g. Hirschsprung's disease or uraemia. In patients with Hirschsprung's disease, non-specific inflammatory colitis may develop into pseudomembranous colitis in the absence of exposure to antimicrobial agents (Brearly *et al.*, 1987). Antimicrobial-associated depression of gastrointestinal motility, which is most pronounced with the lipid soluble lincosamides, may contribute to the development of antimicrobial-associated colitis (Lees and Percy, 1981, Percy and Christensen, 1985).

1.8 Clinical signs and mortality rate of pseudomembranous colitis

Antimicrobial-associated colitis has been defined as a severe, infectious disease, which has symptoms ranging from self-limiting diarrhoea to severe colitis, with or without the formation of intestinal pseudomembranes, which occurs 4-10 days after the commencement of antimicrobial therapy (Aronsson *et al.*, 1982, Clarke *et al.*, 1990a, Dhawan and Thadepalli, 1982, Leung *et al.*, 1985, Levine and Lamont, 1982, Guandalini *et al.*, 1988, Meyer-Kawohl and Bockemuhl, 1986, Mittermayer, 1989, Möllby *et al.*, 1985, Ramirez-Ronda and Sandford, 1975, Trnka and Lamont, 1984). Pseudomembranous colitis is characterized by the development of whitish exudative lymphoid plaques (pseudomembranes) attached to the large intestinal mucosa (Pesce *et al.*, 1984, Saco *et al.*, 1981, Trnka and Lamont, 1984).

A wide variety of clinical signs have been associated with colitis due to *C. difficile* infection. The clinical signs of colitis include abdominal pain (Clarke *et al.*, 1990a, Dhawan and Thadepalli, 1982, Griffin *et al.*, 1990, Leung *et al.*, 1985, McKinley et al., 1982, Machida *et al.*, 1989, Mittermayer, 1989, Mueller and Benowitz, 1989, Ramirez-Ronda and Sandford, 1975), acute onset watery diarrhoea with or without fever (Dhawan and Thadepalli, 1982, Griffin *et al.*, 1990, Hori *et al.*, 1989, Leung *et al.*, 1985, Mittermayer, 1989), pseudomembrane formation (Slagle and Boggs, 1976), intestinal inflammation (Slagle and Boggs, 1976), loss of appetite (Machida *et al.*, 1989), leucocytosis (McKinley *et al.*, 1982, Mittermayer, 1989) and hypoproteinaemia (Buts *et al.*, 1977, Leung *et al.*, 1985). Rybolt *et al.* (1989) demonstrated high concentrations of the serum protein α-1-antitrypsin in stool samples from patients with *C. difficile* infection, and correlated serum protein loss into the gastrointestinal tract with the severity of *C. difficile* infection. The possibility of *C. difficile* colitis should be considered in patients with signs similar to those of intestinal obstruction (Clarke *et al.*, 1990a) or with atypical symptoms of food poisoning (Borriello *et al.*, 1984). Moreover, Drapkin *et al.* (1985) concluded that 5.2% of patients that presented with signs of acute abdominal disease (*e. g.* acute peritonitis, peritoneal irritation and leucocytosis) had *C. difficile* colitis.

The mortality rate of *C. difficile* colitis has ranged from 20% (Zweiner *et al.*, 1989), 23% (Church and Fazio, 1986), or 24% (Hori *et al.*, 1989), to around 30% (Guandalini *et al.*,

1988).

Antimicrobial-associated pseudomembranous colitis should be included in the differential diagnosis when there is a clinical history of recent antimicrobial therapy (Hakkal, 1976, McKinley *et al.*, 1982, Trnka and Lamont, 1984, O'Connor, 1981).

1.9 Diagnosis of pseudomembranous colitis and *C. difficile* infection

A diagnosis of *C. difficile* colitis is made by the endoscopic/sigmoidoscopic demonstration of intestinal pseudomembrane formation (Meyer-Kawohl and Bockemuhl, 1986, McKinley *et al.*, 1982, Mittermayer, 1989, Ramirez-Ronda and Sandford, 1975, Trnka and Lamont, 1984), isolation of the organism (Borriello *et al.*, 1984, Clarke *et al.*, 1990a, Meyer-Kawohl and Bockemuhl, 1986, Mittermayer, 1989) and demonstration of toxin production (Borriello *et al.*, 1984, Meyer-Kawohl and Bockemuhl, 1986, McKinley *et al.*, 1982, Mittermayer, 1989).

Diagnostic imaging techniques, other than endoscopy, have been used to demonstrate the pathognomonic intestinal pseudomembranes. However, the positive contrast radiographic findings of ulceration, mucosal oedema and excessive mucous excretion are insufficient to differentiate antimicrobial-associated pseudomembranous colitis from other inflammatory diseases of the gastrointestinal tract (Hakkal, 1976).

There are a variety of techniques, of varying sensitivity and specificity, available for the demonstration of *C. difficile* infection. Isolation of *C. difficile* using selective media and stringent anaerobic environmental conditions is a sensitive (89%) means of demonstrating the presence of *C. difficile* however it is only 74% specific (Walker *et al.*, 1986). *Clostridium difficile* colitis cannot be diagnosed by the demonstration of the organism alone, since there are strains of low virulence and the development of the disease depends on toxin production. Enzyme-linked immunosorbent assay (ELISA) is a sensitive way of detecting cytotoxin and enterotoxin production. The specificity of the cytotoxin ELISA is low (56%), compared to the enterotoxin ELISA (100%), due to cross-reaction with cytotoxins produced by other *Clostridium spp.*, such as *C. sordelli* (Walker *et al.*, 1986). The microtitre cytotoxicity assay provides a sensitive (78%) and specific (95%) means of demonstrating the presence of *C. difficile*, whereas the tube cytotoxin assay is less specific (79%) (Walker *et al.*, 1986). In 93% of cases of pseudomembranous colitis, a fluorescent antibody test agreed with culture and toxin demonstration for *C. difficile* (Larson *et al.*, 1982).

1.10 Prevention and treatment of pseudomembranous colitis in man

The development of diarrhoea, following the administration of clindamycin, should not prevent the use of this reliable and well tested antimicrobial agent in the treatment of

anaerobic infections since, in a significant number of patients, diarrhoea ceases as soon as drug therapy is discontinued (Dhawan and Thadepalli, 1982, Spiller *et al.*, 1984). The judicious selection and use of antimicrobial agents may reduce the incidence of antimicrobial-associated colitis (Yarinsky and Wheeler, 1990). The importance of appropriate dose rates and regimes have been emphasized. In addition, it has been shown that the duration of antimicrobial prophylaxis should be minimized to reduce the risk of developing *C. difficile* colitis (Clarke *et al.*, 1990a).

Clostridium difficile is an infectious organism therefore patients with *C. difficile* colitis should be isolated to reduce the risk of cross-infection (Leung *et al.*, 1985). The environment is an important source of *C. difficile* infection. Hypochlorite (1600 ppm available chlorine, pH 7.6) was effective in eliminating *C. difficile* from a hospital environment (Kaatz *et al.*, 1988).

Generally, *C. difficile* colitis is cured easily, but it is important to determine patients at high risk, such as those with severe underlying disease or primary or secondary faecal stasis (Colatutto *et al.*, 1989). In addition, some cases of pseudomembranous colitis resolve rapidly following the discontinuation of treatment with the causative antimicrobial agent (Burbige and Radigan, 1987, Church and Fazio, 1986, Hecht and Olinger, 1989, Keshavarzian *et al.*, 1984, Meyer-Kawohl and Bockemuhl, 1986, Ramirez-Ronda and Sandford, 1975, Saco *et al.*, 1981). Generally, patients with culture positive, toxin negative *C. difficile* infection recover without specific treatment (McKinley *et al.*, 1982).

A number of antimicrobial agents have been used to treat *C. difficile* infection. Vancomycin, a narrow spectrum (Gram positive) antimicrobial agent which inhibits bacterial cell wall formation, has been used to treat methicillin-resistant staphylococcal infections and antimicrobial-associated colitis due to *C. difficile* infection (Cheung and Di Piro, 1986, Griffith, 1981, Hermans and Wilhelm, 1987, Levine and Lamont, 1982, McKinley *et al.*, 1982, Saco *et al.*, 1981, Silva *et al.*, 1981). Treatment of pseudomembranous colitis with vancomycin is effective but 10-15% of cases may relapse (Levine and Lamont, 1982). The antibacterial spectrum of metronidazole is limited to microaerophilic and anaerobic bacteria. Oral administration of metronidazole has been used to treat antimicrobial-associated colitis and, in addition, therapy with metronidazole is much cheaper than the standard treatment with oral vancomycin (Bolton and Culshaw, 1986, Briceland *et al.*, 1988, Hecht and Olinger, 1989). The rifamycins, which inhibit bacterial nucleic acid synthesis, are active against Gram positive and some Gram negative bacteria and *Mycobacterium tuberculosis*. The administration of the rifamycin, rifaximin, by nasogastric tube to patients with severe colitis and bacterial superinfection, was highly effective clinically, and there were no reports of local or systemic side effects (Alvisi *et al.*, 1987). In addition, other agents which inhibit

bacterial cell wall synthesis and have activity against Gram positive bacteria have been used to treat antimicrobial-associated colitis due to *C. difficile* infection. These include the cephalosporin ceftizoxime (Iwai *et al.*, 1982), fusidic acid (Canzi *et al.*, 1987), and bacitracin (Trnka and Lamont, 1984).

A range of other agents and supportive measures have been used in the treatment of antimicrobial-associated colitis. Cholestyramine and colestipol are strongly basic anion-exchange resins which have been used in the treatment of antimicrobial-associated colitis. Cholestyramine and colestipol are lipid regulating agents which exchange chloride ions for the anions of bile salts within the gastrointestinal lumen, prevent their absorption from the gastrointestinal tract, and lead to an overall reduction in serum cholesterol, and in particular low density lipoprotein, concentrations. The advantage of cholestyramine and colestipol in the treatment of antimicrobial-associated colitis have been attributed to the fact that they bind to *C. difficile* toxins (Chang *et al.*, 1978, Mogg *et al.*, 1982). Fluid and electrolyte replacement therapy have been used to restore a proper fluid and electrolyte balance (Meyer-Kawohl and Bockemuhl, 1986, Saco *et al.*, 1981). Surawicz *et al.* (1989) noted that the administration of the non-pathogenic yeast *Saccharomyces boulardii* reduced the incidence of antimicrobial-associated diarrhoea in hospitalized patients. In some refractory cases of pseudomembranous colitis, medical treatment is insufficient, and surgical intervention, *e. g.* colectomy, has been of benefit in some of these cases (Boyd and Den Besten, 1976, Eriksson *et al.*, 1982, McKinley *et al.*, 1982).

1.11 Animal models of antimicrobial-associated colitis

A number of different animal models have been used in the diagnosis and evaluation of treatment of antimicrobial-associated colitis. The majority of antimicrobial agents, which result in the development of antimicrobial-associated colitis in man, produce disease in hamsters (Boon and Beale, 1985). The hamster model has been regarded as a sensitive indicator of the antimicrobial agents likely to cause antimicrobial-associated colitis in treated patients since *C. difficile* infection is common in the hamster (Boon and Beale, 1985). Hawkins *et al.* (1984) reported that 13% of hamsters had demonstrable *C. difficile* infection prior to antimicrobial administration. Uninfected and untreated hamsters may acquire *C. difficile* infection either by cross-infection or from the environment (Hawkins *et al.*, 1984, O'Connor *et al.*, 1981). Rehg and Lu (1982) reported *C. difficile* typhilitis in the hamster that was not associated with antimicrobial administration. The ß-lactam antimicrobial agents vary in their ability to cause colitis and death in the hamster-model of *C. difficile* colitis (Weinberg *et al.*, 1986). For example, azotreonam is quite safe following intraperitoneal or oral administration, whereas ceftazidine is only safe to use parenterally, and cefoperazone and latamoxef result in caecitis and death following parenteral administration (Weinberg *et*

al., 1986). Fatal *C. difficile* colitis occurs within a few days of treating hamsters with other ß-lactam antimicrobial agents, including ampicillin (Larson and Borriello, 1990), flucloxacillin (Larson and Borriello, 1990), cefoxitin (Boon and Beale, 1985, Dubini, 1988) ceftazidine (Weinberg *et al.*, 1986), and cefuroxime (Larson and Borriello, 1990). Parenteral administration of latamoxef and cefaperazone to hamsters resulted in marked changes in the anaerobic caecal microflora and the growth of *C. difficile* (Weinberg *et al.*, 1986). Administration of the macrolide N-formimidoyl thienamycin to hamsters resulted in the development of colitis with a mortality rate of 75% (Hawkins *et al.*, 1984). Clindamycin treatment of hamsters resulted in the development of rapidly fatal colitis (Boon and Beale, 1985, Dubini, 1988, Larson and Borriello, 1990, Weinberg *et al.*, 1986). Moreover, a persistent loss of resistance to *C. difficile* colonization, a type of post-antibiotic effect, was reported in hamsters which developed non-fatal colitis following administration of clindamycin. O'Connor *et al.* (1981) reproduced the typical clinical and morphological changes associated with colitis following administration of a rifamycin antimicrobial agent, rifampicin, to hamsters.

Fekety *et al.* (1979) prevented clindamycin-induced *C. difficile* colitis in hamsters by the oral administration of either vancomycin or metronidazole. The acidic lipopeptide antimicrobial agent LY146032, which inhibits the biosynthesis of bacterial cell wall peptidoglycan, delayed death in the hamster model of pseudomembranous colitis at a dose rate of 0.05 mg/day; equivalent protection with vancomycin would require administration at 100 times the dose rate, *i. e.* 5 mg/day (Dong *et al.*, 1987). The survival of hamsters was prolonged significantly by the administration of cholestyramine but the development of colitis and death were not prevented (Fekety *et al.*, 1979). Treatment with corticosteroids or an atropine/diphenoxylate combination did not alter the course of clindamycin-induced colitis in the hamster (Fekety *et al.*, 1979).

A similar phenomenon occurs in other species. Guinea pigs are susceptible to *C. difficile* infection and the high incidence of antimicrobial-associated colitis restricts the use of many antimicrobial agents in this species (Fritz *et al.*, 1987). George *et al.* (1979) and Lowe *et al.* (1980) reported *C. difficile* associated caecitis in guinea pigs exposed to penicillin, and caecal filtrates from penicillin treated guinea pigs which developed colitis contained *C. difficile* toxin (George *et al.*, 1979, Rothman, 1981). Vancomycin treatment of guinea pigs, with penicillin-induced *C. difficile* colitis, increased the survival time and decreased the number of deaths (Rothman, 1981). Similarly, antimicrobial-associated colitis has been reported in the rabbit. Guandalini *et al.* (1988) reported the development of *C. difficile* colitis in rabbits following oral administration of ampicillin. In addition, administration of clindamycin to rabbits has been shown to alter the quantitative and qualitative composition of the obligate anaerobic microflora (Poliak *et al.*, 1983).

1.12 Colitis in the equine

In the horse, colitis has been described using a variety of different names, such as equine non-parasitic diarrhoea (Nielsen and Vibe-Petersen, 1979), acute diarrhoea (Wierup and Di Pietro, 1981), acute equine colitis (Roberts, 1990, Whitlock, 1986), colitis 'X' (Harries and Strother, 1969, Kelly, 1972, Onderdonk, 1985, Prescott *et al.*, 1988, Vaughan, 1973, Wierup and Di Pietro, 1981), exhaustion shock (Harries and Strother, 1969, Rooney *et al.*, 1963, Onderdonk, 1985), or fatal idiopathic colitis (Prescott *et al.*, 1988). Proudman (1992) found that 1% of equine colic cases were due to colitis and that in general these cases were fatal.

The cause of equine colitis is unknown, but specific causes have been demonstrated. Recently, equine intestinal clostridiosis was described as an enterotoxaemia caused by high numbers of *C. perfringens* in the gastrointestinal tract (Pearson *et al.*, 1986, Schiefer, 1981, Sims *et al.*, 1985, Wierup and DiPietro, 1981). The colitis 'X' syndrome was believed to be related to a toxaemia brought on by the absorption of endotoxins from autolysing Gram negative bacteria in the gastrointestinal tract (Harries and Strother, 1969, Kelly, 1972).

In the equine a diagnosis of colitis describes a syndrome with a wide range of different causative agents. Colitis-associated diarrhoea has been reported as a sequel to a number of well recognized infections in the horse, including parasitic infections such as migrating strongyle larvae (Blackwell, 1973, Breider *et al.*, 1985, Jasko and Roth, 1984), and bacterial infections such as *Salmonella spp.*, including *Salmonella typhimurium* (Dorn *et al.*, 1975, Goetz and Coffman, 1984, Manahan, 1970, Slocombe and Slauson, 1988, Whitlock, 1986), and *Ehrlichia risticii* infection (Cordes *et al.*, 1986, Palmer *et al.*, 1986, Van Der Kolk and De Groot, 1991). In addition, equine colitis-associated diarrhoea has been recorded following infection with bacteria isolated less commonly, including *E. coli* (Kelly, 1972, Manahan, 1970), *Staphylococcus spp.* (Manahan, 1970), *Mycobacterium avium* (Buergelt *et al.*, 1988, Cline *et al.*, 1991), *Clostridium spp.*, including *C. difficile* (Jones *et al.*, 1987, Jones *et al.*, 1988) and *C. cadaveris* (Prescott *et al.*, 1988) and *Acinetobacter calcoaceticus* (Dickie and Regnier, 1978). In addition, other organisms, such as *Trichomonas*-type protozoa (Manahan, 1970), *Giardia*-type protozoa (Manahan, 1970), *Geotrichum*-type fungi (Manahan, 1970), *Histoplasma spp.* (Dade *et al.*, 1973, Goetz and Coffman, 1984), and the ciliate *Polymorphella ampulla* (Gregory *et al.*, 1986), have been reported as a cause of colitis-associated diarrhoea in the equine.

1.13 Antimicrobial agents associated with colitis in the equine

In the equine the development of colitis has been associated with the administration of antimicrobial agents. Diarrhoea has developed following the administration of the penicillins (Baggot *et al.*, 1990, English and Roberts, 1983, Roberts, 1990), including penicillin V (Baggot *et al.*, 1990, Ducharme *et al.*, 1983), chloramphenicol (Nielsen and Vibe-Petersen, 1979), the macrolides spiramycin (Nielsen and Vibe-Petersen, 1979), erythromycin (Baggot and Prescott, 1987, Roberts, 1990) and tylosin (Roberts, 1990), lincomycin and clindamycin (Raisbeck *et al.*, 1981, Prescott *et al.*, 1988), metronidazole (Sweeney *et al.*, 1991), and the potentiated sulphonamides (Murray, 1988b, Roberts, 1990) The adverse reaction to the tetracyclines should be familiar to all equine clinicians (Roberts and English, 1979, Whitlock, 1990). Diarrhoea has been reported following administration of oxytetracycline (Andersson *et al.*, 1971, Baker and Leyland, 1973, Cook, 1973, Kuiper and Franken, 1980, Mackellar *et al.*, 1973, Nielsen and Vibe-Petersen 1979, Owen, 1975, Swerczek, 1979) and chlortetracycline (Cook, 1973).

One of the most common causes of adverse gastrointestinal reactions to antimicrobial agents in the equine appears to be the feeding of contaminated or inappropriate feedstuffs. Diarrhoea was reported following the oral administration of a premix containing oxytetracycline, sulphadimethoxine and furazolidone and following oral administration of an oxytetracycline and sulphadiazine premix (Ruckebusch, 1983). Rollinson *et al.* (1987) reported salinomycin poisoning, in horses fed contaminated feed, similar to that seen following administration of another ionophore antimicrobial agent, monensin.

1.14 Other factors involved in the development of equine colitis

The administration of other therapeutic agents, including anthelmintics and non-steroidal anti-inflammatory drugs, has resulted in the development of diarrhoea in horses (Karcher *et al.*, 1990, Nielsen and Vibe-Petersen, 1979, Simmons *et al.*, 1990).

Stress, such as a change of diet, strenuous work, abdominal pain, transport, hospitalization, general anaesthesia and abdominal surgery, has initiated clinical enteritis possibly due to an increased susceptibility to intestinal infections (Baker and Leyland, 1973, Clarke *et al.*, 1990b, Cook, 1973, Harries and Strother, 1969, Mackellar *et al.*, 1973, Manahan, 1970, Nielsen and Vibe-Petersen, 1979, Owen, 1975, Palmer *et al.*, 1985, Rooney *et al.*, 1963, Schiefer, 1981, Swerczek, 1979, Vaughan, 1973, Wierup and DiPietro, 1981, Wilson, 1979). Proudman (1992) found that 43% of cases of spasmodic colic were associated with stress, such as drug administration, transportation, a management or weather change, surgery and an unusual feed intake.

The alterations in the gastrointestinal tract that occur as a result of so-called stress may be due to exposure to different organisms, or to a reduction in feed and water intake, even if this is only temporary. In the pig, the coliform flora is complex and a turnover of biotypes has been associated with changes in pen occupancy and diet (Hedges and Linton, 1988). In the pony, an abrupt change in diet from hay to concentrates resulted in an increase in the total number of viable anaerobic bacteria and an alteration in the luminal pH in the caecum, similar to those seen in the bovine rumen under similar feeding conditions (Goodson *et al.*, 1988).

Hird *et al.* (1986) reported that 38.8% (83/214) of cases of equine salmonellosis were primary infections whereas 61.2% (131/214) were hospital acquired infections. The odds for developing salmonellosis were higher if the animal was admitted as an emergency, or if treatment involved nasogastric intubation, intravenous catheterization, rectal examination, surgery especially invasive abdominal surgery or enterotomy, inhalational anaesthesia and parenteral or oral antimicrobial administration. It was suggested that horses with intestinal disturbances were more susceptible to *Salmonella spp.* infection than other horses, although there was a slight bias in that it was more likely for samples to be submitted for bacteriological examination from these animals.

1.15 Clinical signs and mortality rate of colitis in the equine

Profuse watery diarrhoea is the commonest sign of colitis in the equine (Roberts, 1990). However, there are a wide spectrum of clinical signs associated with the build up of fluid in the intestinal lumen. Consequently, equine colitis presents with clinical signs ranging from peracute, acute or subacute colic to chronic mild diarrhoea which usually occur within approximately 3 days of commencing drug administration (Andersson *et al.*, 1971, Baker and Leyland, 1973, Cook, 1973, English and Roberts, 1983, Harries and Strother, 1969, Karcher *et al.*, 1990, Kuiper and Franken, 1980, Manahan, 1970, Nielsen and Vibe-Petersen, 1979, Parry, 1983, Rooney *et al.*, 1963, Schiefer, 1981, Whitlock, 1986, Wierup and DiPietro, 1981, Wilson, 1979). However, the onset of diarrhoea may be delayed for some weeks after the cessation of antimicrobial treatment (Cook, 1973, Ruckebusch, 1983). Thus the clinical picture can be of sudden onset explosive diarrhoea resulting in dehydration which, in the absence of appropriate treatment, may lead to the development of shock, disseminated intravascular coagulation and death within a few days. At the opposite end of the spectrum, depression, anorexia and mild diarrhoea and, in chronic cases, hypoproteinaemia and weight loss may be the only clinical signs.

A guarded prognosis should be given to horses with colitis (Nielsen and Vibe-Petersen, 1979). The mortality rate of horses with acute colitis was 83% (Nielsen and Vibe-Petersen, 1979), although the longer the animals live the greater the survival rate (subacute cases of

colitis had a mortality rate of 45%).

Acute oxytetracycline-induced diarrhoea, intestinal clostridiosis and the colitis 'X' syndrome are not dissimilar clinically or at necropsy (English and Roberts, 1983). At *post mortem* examination there are a wide range of pathological findings and not surprisingly the most striking findings are in the gastrointestinal tract. Pathological findings may be restricted to the large intestine, *e. g.* typhlocolitis (inflammation of the caecum and colon) (Harries and Strother, 1969, Prescott *et al.*, 1988, Rooney *et al.*, 1963), or may extend from the duodenum to the colon (Schiefer, 1981). Generally, the intestinal lumen is filled with watery contents, and there is mucosal oedema, and mucosal necrosis with or without ulcers (Harries and Strother, 1969, Prescott *et al.*, 1988, Rooney *et al.*, 1963, Schiefer *et al.*, 1981).

1.16 Diagnosis of colitis in the equine

A diagnosis of colitis has been applied to a wide variety of infections with similar clinical signs, such as salmonellosis, intestinal clostridiosis, and strongyle larval migrans. Consequently, there is no definitive technique for diagnosing of colitis in the equine. In many cases, the clinical course can be so rapid that a diagnosis is made at *post mortem* examination only. In more protracted cases, standard techniques of bacterial isolation can be used. However, there are a wide range of causative organisms many of which are present in normal faeces and, in addition, repeated sampling may be required to demonstrate pathogenic organisms, such as *Salmonella spp.*.

There is a paucity of information on the microbial population that inhabits the gastrointestinal tract of the horse (Mackie and Wilkins, 1988). It has been suggested that a diagnosis of equine intestinal clostridiosis can be based upon the isolation and enumeration of *C. perfringens* from intestinal contents or faeces (Wierup and Di Pietro, 1981). However, studies of the faecal flora in man and animals, after antimicrobial therapy, have suffered from a lack of quantitative techniques to cope with the enormous variations in the number and the type of organism present in the gastrointestinal microflora (Trnka and Lamont, 1984). In addition, there can be differences in the flora of animals managed under different conditions (Wierup and Di Pietro, 1981). It may be that, given sufficient information on the number of bacteria in the normal gastrointestinal tract, techniques that enumerate bacteria could be used in the diagnosis of colitis in the equine. It should be remembered that these techniques reflect luminal and not mucosal bacterial populations, although it has been shown that, in the horse, there is a close relationship between the number of viable luminal and mucosal bacteria in the gastrointestinal tract (Mackie and Wilkins, 1988).

1.17 Prevention and treatment of colitis in the equine

It would be prudent to avoid the administration of antimicrobial agents which have been associated with the development of potentially life-threatening diarrhoea in other species, particularly where there is not specific information on their use in the equine. Nielsen and Vibe-Petersen (1979) recommended that the administration of chloramphenicol, the lincosamides and the tetracyclines should be avoided in horses. In addition, all cases of colitis should be treated with care, even where a specific pathogen has been isolated, since antimicrobial therapy may exacerbate the condition (Schiefer, 1981).

Obviously, if antimicrobial-associated colitis is suspected, the administration of the causal antimicrobial agent should be stopped immediately. Fluid and electrolyte loss into the gastrointestinal lumen, and absorption of enterotoxin or endotoxin through the intestinal mucosa, produce systemic fluid and electrolyte imbalances and lead to the development of life-threatening hypovolaemic or endotoxic shock. Therefore, the treatment of equine colitis aims to correct the body fluid and electrolyte balances by vigorous fluid and electrolyte replacement therapy (Harries and Strother, 1969, Manahan, 1970, Nielsen and Vibe-Petersen, 1979, Roberts, 1990, Vaughan, 1973). In addition, a variety of different agents have been recommended for administration to horses with colitis-associated diarrhoea. These include intestinal protectants, antimicrobial agents, analgesics, anti-diarrhoeal agents, sodium bicarbonate and dietary changes (Harries and Strother, 1969, Nielsen and Vibe-Petersen, 1979, Roberts, 1990).

1.18 The commensal gastrointestinal microflora and toxins

Monogastric animals have a modification of the lower intestinal tract, inhabited by symbiotic microorganisms, whose function is to breakdown some of the fibrous feed components since, in general, the animals lack the enzymes that degrade cellulose. In the horse, the caecum and colon have been enlarged to accommodate microbial colonization and fermentation.

At birth the large intestine is sterile and it acquires an anaerobic microflora gradually (Murray *et al.*, 1987). A wide variety of potentially pathogenic bacteria are found in the gastrointestinal tract (Strachan, 1983, White and Prior, 1982), including coliforms, streptococci and *Clostridium spp.*, and these occur in varying numbers in the faeces from pigs, chickens, sheep, calves, dogs, cows, horses and man (Havelaar *et al.*, 1986). Multiplication of bacteria in the gastrointestinal lumen can occur when an obstruction or blood are present, and when the animal's immune defences are lowered (Strachan, 1983). Moreover, stress results in an increase in the number of viable coliforms and reduction in the

number of viable lactobacilli in the gastrointestinal tract, and may reduce VFA concentrations due to a depression of the anaerobic microflora (Sogaard, 1986).

A number of different bacterial species produce disease by the production of enterotoxins, cytotoxins or endotoxins. Enterotoxins and cytotoxins are preformed toxins produced by a number of Gram positive and Gram negative bacteria including *Clostridium spp.*, toxin-producing staphylococci and enterotoxigenic *E. coli*. *Clostridium difficile* produces both enterotoxin and cytotoxin which damage the intestinal mucosa and result in the development of pseudomembranous colitis in man. An enterotoxaemia produced by the overgrowth of toxin producing *C. perfringens* in the gastrointestinal tract has been described in the horse and in foals (Pearson *et al.*, 1986, Wierup and Di Pietro, 1981). In addition, diarrhoea due to *C. difficile* infection has been reported in foals (Jones *et al.*, 1987, Jones *et al.*, 1988).

Endotoxin is the lipopolysaccharide outer cell wall of Gram negative bacteria, such as *E. coli*, and *Salmonella*, *Klebsiella*, *Proteus* and *Pseudomonas spp.*. Endotoxin has been implicated in the pathogenesis of a variety of diseases in the equine, including laminitis, septic shock, colitis 'X' and colic (Burrows, 1981c, King and Gerring, 1988, Muir, 1987). Endotoxin is present in the normal gastrointestinal lumen and the intestinal mucosa generally represents an effective barrier (King and Gerring, 1988, Moore and Morris, 1992). Hence, there is a concentration gradient of endotoxin from the gastrointestinal lumen *via* the peritoneal cavity to the systemic circulation, and there are low concentrations of endotoxin in the peritoneal cavity and systemic circulation of normal horses. However, the absorption of large quantities of endotoxin into the peritoneal cavity, and hence into the systemic circulation, may occur when the gastrointestinal wall is damaged, *e. g.* in intestinal ischaemia as a sequel to intestinal volvulus or incarceration. An experimental increase in circulating endotoxin concentrations produced a variety of clinical signs in the pony, including colic, lactic acidosis and diarrhoea (Moore *et al.*, 1981). In addition, experimental acute *E. coli* endotoxaemia in ponies resulted in a variety of blood biochemical and haematological alterations, including an increase in the packed cell volume, blood glucose and blood lactate concentrations and a reduction in the plasma bicarbonate ion concentration, pH and white blood cell count (Burrows, 1981a, b, c, Green and Adams, 1992, Moore and Morris, 1992, Morris *et al.*, 1986, Stadler and Van Amstel, 1989, Ward *et al.*, 1987). In clinical cases, an increase in the plasma endotoxin concentration has been correlated with an increase in heart rate and packed cell volume, and with death by King and Gerring (1988).

1.19 Microbial fermentation in the gastrointestinal tract

The extensive fermentation of polysaccharides and the production of SCFA in the hind gut is a common feature of all mammals, although the hindgut volume varies considerably between carnivores, omnivores and herbivores (Alexander, 1971, Argenzio *et al.*, 1974, Bruorton *et*

al., 1991, Bugaut, 1987, Cummings *et al.*, 1976, Hintz, 1975, Stevens *et al.*, 1986, Weaver *et al.*, 1988). The role of SCFA in the nutrition and health of the large intestine is well recognized in man (Fleming and Arce, 1986) and in ruminants (Czerkawski, 1986).

The metabolic products of bacterial fermentation, SCFA, make a major contribution to the energy metabolism in animals (Rechkemmer *et al.*, 1988, Tisserand, 1989). The highest SCFA concentrations have been recorded in the forestomachs of ruminants and in the large intestine of all mammals (Bugaut, 1987). The biochemical activities and microbial composition of the microflora reflect the anaerobic environment of the gastrointestinal tract (Mackie and Wilkins, 1988) and the microbial metabolism in the lower intestine of monogastric animals resembles the reactions that occur in the rumen (Miller and Wolin, 1979, Prins, 1987).

Most non-sporing anaerobes in the gastrointestinal tract ferment carbohydrates according to the Embden-Meyerhof-Parnas scheme (Miller and Wolin, 1979). Non-sporing anaerobic bacteria in the gastrointestinal tract decarboxylate pyruvate to acetyl coenzyme A (the 2 carbon radical of acetate) and carbon dioxide, with the concomitant reduction of a low-potential electron acceptor or the reduced form of nicotinamide adenine dinucleotide (NADH), and the formation of hydrogen (Coffman, 1975a, b, Miller and Wolin, 1979). Lactate is produced by the oxidation of the NADH formed during glycolysis (Miller and Wolin, 1979). Acetate, ethanol or butyrate are formed from acetyl coenzyme A, and the carboxylation of pyruvate or phosphoenolpyruvate produces oxaloacetate which is reduced, eventually, to succinate, a precursor of propionate. Isobutyrate, isovalerate, valerate and hexanoate are produced by valine, leucine and polypeptide degradation, respectively (Mortensen *et al.*, 1988, Zarling and Ruchim, 1987).

1.20 Lactic acid

Gastrointestinal bacteria produce both D- and L-lactic acid, the latter being the endogenous molecular species in mammals, in a process which is dependent, partially, on thiamine (Coffman, 1975a, b). Faecal coliform bacteria in cattle, horses and red pandas produced D-lactate, acetate and ethanol in 1% glucose broth *in vitro* (Slyter and Rumsey, 1991). Lactic acid in the intestinal contents of ruminants is derived from lactate in feed and from readily fermentable carbohydrate (Wolffram *et al.*, 1988). Substantial amounts of lactic acid are produced by bacterial fermentation in the stomach of the adult horse and these are either absorbed in the small intestine or fermented in the colon (Alexander, 1971, Argenzio *et al.*, 1974). Sodium ions stimulate L-lactate uptake, in isolated bovine brush border membrane vesicles, whereas acetate, propionate and butyrate inhibit sodium ion dependent L-lactate uptake (Wolffram *et al.*, 1988).

Lactic acid metabolism can be affected by feeding. Dietary protein content did not affect the blood lactate concentrations in horses, but the lactate to pyruvate concentration ratio was higher when a low protein (9%) diet was fed compared with a high (18.5%) protein diet (Miller-Graber *et al.*, 1991). As a result Miller-Graber *et al.* (1991) suggested that the conversion of lactate to pyruvate may be influenced by the composition of the diet in the horse.

In the bovine rumen and in the equine caecum, the lactic acid concentrations increase following the fermentation of starch and soluble sugars, *e. g.* following carbohydrate overload (Coffman, 1975a, b, Wernery and Wensvoort, 1992). Experimentally induced lactic acidosis (by intraruminal glucose administration) resulted in a typical reduction in ruminal pH and a concurrent increase in L- and D-lactic acid concentrations (Muir *et al.*, 1980, Nagaraja *et al.*, 1982). Following carbohydrate overload in the horse, there was an increase in the number of viable lactate producing bacteria and a reduction in the number of viable Gram negative bacteria (Garner *et al.*, 1978), an increase in the caecal endotoxin and lactic acid concentrations (Moore *et al.*, 1979), and a reduction in the pH of caecal samples (Moore *et al.*, 1979, Garner *et al.*, 1978).

Systemic lactic acidosis is characterized by metabolic acidosis, due to hydrogen ion accumulation in blood, and a blood lactate concentration of at least 5 mM (Arieff *et al.*, 1982). An increase in blood D- and L-lactate concentrations resulted in the development of metabolic acidosis with a high anion gap and changes in the plasma potassium ion and serum inorganic phosphate concentrations in ponies (Gossett *et al.*, 1987, Gossett *et al.*, 1990a, b). Moore *et al.* (1976) showed a correlation between increased blood lactate concentrations and a reduction in the percentage survival in horses with colic, and suggested that blood lactate concentrations were a good prognostic indicator in horses with colic.

1.21 Volatile fatty acids

The main end products of saccharide fermentation are the VFAs, acetate, propionate and butyrate, and these are the major anions in the large intestine (Argenzio, 1981, Czerkawski, 1986, Mortensen *et al.*, 1988, Weaver *et al.*, 1988, Zarling and Ruchim, 1987). In the equine, 50% of the ingested soluble carbohydrate is fermented to produce VFAs in the caecum and colon (Argenzio, 1975, Murray, 1988a). Bonhomme-Florentin (1988) showed that components of the horse caecal contents break down plant structural polysaccharides (hemi-cellulose and pectin) *via* polysaccharide depolymerases and glycoside hydrolases.

Diet influences VFA production in the rumen and human colon (Mortensen *et al.*, 1988, Prins, 1987). Addition of a yeast culture supplement to the diet of Holstein cows has been

shown to reduce the ruminal pH, ammonia concentration, molar proportions of acetate and isovalerate and the acetate to propionate concentration ratio and to increase the molar proportions of propionate and valerate, but not to affect the proportions of isobutyrate, butyrate, or the total VFA or viable yeast concentrations in ruminal fluid (Harrison *et al.*, 1988). In the rat, the incorporation of fibre and crude potato starch in the diet resulted in a significant enlargement of the caecum and an increase in VFA concentrations and an increase in the pool of acetate, propionate and butyrate (Mazur *et al.*, 1990). It has been suggested that, in the horse, the concentration ratio of acetate to propionate is influenced by the level of soluble carbohydrate in the diet (Hintz, 1975). However, Wolter *et al.* (1980) fed a complete pelleted diet to ponies and rabbits and found that there were no statistically significant differences in the dry matter content, starch digestion and the molar ratio of acetate, propionate and butyrate in the gastrointestinal tract.

In the rabbit, a daily meal was followed by an increase in the VFA concentrations and a reduction in the pH of caecal contents (Fioramonti and Ruckebusch, 1976). In the miniature swine, digesta entering the caecum produced an increase in SCFA concentrations and a reduction in pH (Fleming *et al.*, 1989). Fluctuations in the VFA concentrations in rabbits and sheep are marked following once daily feeding, compared with *ad libitum* feeding and twice daily feeding, respectively (Parker and McMillan, 1976, Van der Walt and Briel, 1976). Similarly, in the equine twice daily feeding of high energy, low carbohydrate forage rations resulted in an increase in the amount of fermentable carbohydrate in the caecum, due to an increase in the intestinal transit rate, and may produce changes in the gastrointestinal microflora and alterations in the intraluminal endotoxin pool (Clarke *et al.*, 1990b).

In the equine, cyclic changes in VFA production and pH have been related to the cyclic changes in gastrointestinal volume which occur in association with cyclic periods of microbial digestion (Argenzio, 1975, Argenzio *et al.*, 1974). Studies on the effect of episodic feeding on the microbial digestion within the equine large intestine have suggested that the fermentation cycle represents a subclinical carbohydrate overload (Clarke *et al.*, 1990b).

In the colon, VFAs mediate the symbiotic existence of the bacteria and the colonic mucosa and play an important role in the nutrition of colonocytes (Roediger, 1980, Sakata, 1987). VFAs are absorbed readily by the simple columnar epithelium of the intestine, but not all the absorbed VFAs reach the plasma due to metabolism in the gastrointestinal wall where they are an important energy source (Argenzio, 1975, Bugaut, 1987, Roediger, 1980). Both acetate and propionate are mitogenic to ovine rumen epithelial cells but are less so than butyrate (Sakata and Tamate, 1979). In the rat, there is a dose dependent, stimulatory effect of VFAs on ileal epithelial cell proliferation, with butyrate being the most active followed by

propionate and then acetate (Sakata, 1987). This effect is independent of the presence of gut bacteria and low luminal pH. The major respiratory fuel in colonocytes is *n*-butyrate (Kripke *et al.*, 1989, Roediger, 1980, Roediger, 1982, Thomsen *et al.*, 1984) and carbon dioxide production, due to butyrate utilization may provide a useful means of examining the functional activity of the colonic mucosa clinically and *in vivo* (Roediger, 1982).

1.22 Mechanisms of absorption and secretion in the large intestine

Transport mechanisms absorb VFAs from the large intestinal lumen to maintain the luminal pH, between 6.8 and 7.2, and the luminal osmolality at 300 milliosmoles/kg (Alexander, 1971, Argenzio *et al.*, 1974, Argenzio and Stevens, 1975, Bugaut, 1987, Murray, 1988a, Rechkemmer *et al.*, 1988, Umesaki *et al.*, 1979). The magnitude of colonic fermentation is dependent upon the load of rapidly fermentable substrate delivered to the resident microflora (Clarke *et al.*, 1990b). Rapid fermentation requires transmural fluid movement, to buffer and dilute the organic acids produced, and, in addition, the induction of renal and humoural mechanisms to maintain systemic homoeostasis (Clarke *et al.*, 1990b). In hindgut fermenting herbivores, the ileal outflow provides the fluid and buffering capacity essential for microbial metabolism (Rechkemmer *et al.*, 1988). However, the buffering capacity of the ileal outflow of the horse is exhausted by the caecum, and the colon has developed an independent means of buffering during periods of rapid fermentation (Alexander, 1971, Argenzio, 1975).

In the equine caecum, during periods of rapid digestion, sodium/hydrogen ion exchange is interrupted, acetate is exchanged for the bicarbonate ion, and net water absorption is abolished (Argenzio, 1975, Argenzio *et al.*, 1977). However, there are no changes in the osmolality of the caecal contents, suggesting that fluid retention and the neutralization of luminal contents may be controlled by neurotransmitters and gastrointestinal hormones. Between periods of rapid fermentation, VFA concentrations fall, water and sodium ions are absorbed, bicarbonate ions are buffered by sodium/hydrogen ion exchange and carbon dioxide is absorbed into the bloodstream (Argenzio and Stevens, 1975, Murray, 1988a).

Most VFAs are absorbed in an undissociated form, but at physiological pH *circa* 99% exist in an ionized form (Argenzio, 1981, Argenzio and Stevens, 1975, Argenzio *et al.*, 1974, Argenzio *et al.*, 1977, Bugaut, 1987, Engelhardt *et al.*, 1989, Murray, 1988a, Umesaki *et al.*, 1979). Carbon dioxide, a product of bacterial fermentation, combines with water to produce hydrogen and bicarbonate ions, and the dissociated VFAs combine with the hydrogen ions to produce undissociated, absorbable VFAs (Bugaut, 1987, Murray, 1988a, Roediger, 1989). In addition, there is a sodium/hydrogen ion exchange. There is an interdependency between VFA absorption and sodium ion absorption, and between VFA absorption and bicarbonate ion secretion in a variety of *genera* (Argenzio, 1975, Argenzio

and Stevens, 1975, Stevens *et al.*, 1986, Titus and Ahearn, 1988, Umesaki *et al.*, 1979). The formation of undissociated VFAs results in the accumulation of bicarbonate ions in the colonic lumen, and a concomitant reduction in the luminal partial pressure of carbon dioxide (Argenzio, 1975, Stevens *et al.*, 1986, Rechkemmer *et al.*, 1988, Umesaki *et al.*, 1979). In addition, bicarbonate ions are secreted in exchange for chloride ions and there is a net accumulation of bicarbonate ions in the gastrointestinal lumen (Argenzio *et al.*, 1977, Edmonds, 1982, Rechkemmer *et al.*, 1988, Umesaki *et al.*, 1979). The ability of the large intestine to secrete bicarbonate ions in exchange for other anions, both chloride and VFAs, may control intraluminal pH by the alkalization of contents (McNeil *et al.*, 1978, Umesaki *et al.*, 1979). In addition, the poor absorption of bicarbonate ions by the colon restricts the absorption of sodium ions (Argenzio, 1975). Potassium and inorganic phosphate ions are absorbed poorly and are found in high concentrations in the distal colon of the horse (Argenzio, 1975).

The absorption of VFAs is rapid, passive, and independent of luminal pH (except at below pH 6.4), due to the constant pH microclimate at the mucosal surface (McNeil *et al.*, 1978, Rechkemmer *et al.*, 1988, Engelhardt *et al.*, 1988). In the large intestine, sodium, bicarbonate and chloride ions are transported against electrochemical gradients (Argenzio, 1975, Argenzio *et al.*, 1977, Edmonds, 1982, Stevens *et al.*, 1986). The energy for the active transport of sodium ions is provided by glucose and by the absorption of acetate (Argenzio, 1975). The human and equine colon absorb sodium ions more slowly than VFAs (Argenzio, 1975, McNeil *et al.*, 1978, Stevens *et al.*, 1986). There is a relationship between the net movement of water and the net production of VFAs, and between net movement of water and the osmolality of the intestinal contents (Argenzio, 1975, Cummings *et al.*, 1976). In addition, there is a relationship between the net movement of water and solutes (Argenzio, 1975, Murray, 1988a).

In the rat colon, absorption of VFAs, such as acetate, propionate, and butyrate, is much greater than absorption of NVFAs, such as succinate and lactate (Umesaki *et al.*, 1979). In the human jejunum, acetate, propionate and butyrate are absorbed rapidly at equal rates in a linear fashion, but the absorption process reaches saturation at higher concentrations (40-50 mM) (Schmitt *et al.*, 1976). Different monocarboxylate anions (pyruvate, acetate, propionate and butyrate) may have different affinities for a common carrier (Lamers, 1975). Acetate, propionate, butyrate, and high concentrations of the bicarbonate ion, stimulate the efflux of pyruvate, probably due to an anionic counter transport mechanism, in intestinal epithelial cells, *in vitro* (Lamers and Hulsmann, 1975). Lamers and Hulsmann (1975) suggested that the lipophilicity and acidic dissociation constants of the monocarboxylic acids may determine the contribution of non-ionic diffusion to the overall transport of SCFAs. Pyruvate transport is likely to be carrier mediated as shown by saturation kinetics, competitive inhibition by

SCFAs and counter transport (Lamers and Hulsmann, 1975).

The large intestine of the horse has a critical function in the storage and absorption of large volumes of water and in general the caecal contents are more fluid than those of the colon (Argenzio 1975, Frape 1983). Thus, the equine caecum is the primary site of net water absorption. Each day in the normal animal, a large volume of fluid, equivalent to the extracellular fluid volume of the animal, circulates between the intestinal lumen and the body (Argenzio, 1978, Argenzio and Stevens, 1975, Argenzio *et al.*, 1974). Horse faeces usually contains 66-76% water (*i. e.* 24-34% dry matter), although ponies and donkeys may void faeces of a drier consistency (Frape 1983). Diet can affect faecal consistency. In man, an increase in dietary wheat fibre results in an increase in faecal weight largely due to a relative reduction in solid content and an increase in water content (Cummings *et al.*, 1976). In the horse, a high grain diet decreases the faecal water content and pelleted foods tend to increase the faecal moisture content (Frape, 1983).

1.23 Abnormalities of the large intestine

Severe diarrhoea and conditions which affect the large intestine, *e. g.* colitis 'X', result in the development of metabolic acidosis due to a loss of sodium and bicarbonate ions into the intestinal lumen, and lactic acidosis and hypercarbia (an increased blood lactate and a base excess) due to anaerobic tissue metabolism (Manahan, 1970, Roberts, 1990, Svendsen *et al.*, 1979, Vaughan, 1973).

The pathogenesis of colitis-associated diarrhoea, described by Argenzio (1975), Argenzio (1978), Murray (1988b) and Roberts (1990), includes the failure of colonic absorption and secretion processes due to abnormal mucosal permeability, abnormal motility, abnormal ion transport and abnormal microbial digestion. Interference with colonic absorption can be due to mucosal damage, a reduction in the fluid transit time, toxins or drugs which alter solute transport, interference with the intercellular hydrostatic pressure gradients due to junctional complex damage, or inhibition of the active transport mechanisms. An increase in net secretion results when there is an alteration in the capillary and tissue pressure dynamics, high luminal osmotic pressure or active electrolyte secretion. Damage to intercellular tight junctions, *e. g.* due to unabsorbed acid, reduces the pressure gradients and makes absorption difficult or impossible. Epithelial damage, such as inflammatory lesions of the colon, reduces mucosal permeability due to loss of specific entry systems. An increase in tissue pressure may increase hydrostatic pressure and result in net intestinal secretion. Primary causes of deranged intestinal motility are uncommon and a primary increase in activity does not increase luminal volume. A reduction in the intestinal transit time or an alteration in secretion or absorption in the small intestine may result in an increase in the load on the colon, a reduction in mucosal contact and malabsorption (Argenzio, 1978, Mohsen *et*

al., 1987).

Intestinal absorption may be affected by antimicrobial agents. In the human jejunum, the absorption of sodium ions and water was inhibited reversibly by therapeutic concentrations of clindamycin (Spiller *et al.*, 1984). A significant impairment of the bicarbonate ion stimulated absorption of sodium ions and water would increase the volume of fluid entering the ileum, but the development of diarrhoea would not be expected unless both the ileal and colonic absorption were impaired. Dobbins *et al.* (1968) demonstrated a morphological brush border alteration associated with malabsorption, and the subsequent development of diarrhoea, following neomycin treatment.

Bacterial infections can affect mucosal transport mechanisms. Guandalini *et al.* (1988) demonstrated that *C. difficile* infection in the rabbit reduced both glucose and amino acid transport in the jejunum and ileum. In the ileum, *C. difficile* increased the conductance and enhanced the permeability to electrolytes with lesser shifts in sodium and chloride ion transport. In the caecum, *C. difficile* infection has profound effects on sodium and chloride ion transport resulting in a net secretion of the chloride ion. In another study, *C. difficile* toxin resulted in bicarbonate ion accumulation and an increase in the pH of the rabbit large intestine (Lima *et al.*, 1988).

An infusion of SCFAs, at concentrations similar to those produced by bacterial fermentation, into rat ileum reduced the stomach to caecum transit time, particularly at high acid concentrations and with short chain lengths (*e. g.* acetic acid) (Richardson *et al.*, 1991). Primary or secondary alterations in SCFA concentrations can produce intestinal disease. An increase in the luminal osmotic pressure may be secondary to luminal acidification or lactic acid production (Argenzio, 1978, Argenzio, 1981, Garner *et al.*, 1978). In pathological and physiological states where there is diminished SCFA oxidation, *e. g.* ulcerative colitis, the absorption of sodium ions is reduced (Roediger, 1989). Either a suppression of or an abnormal increase in the gastrointestinal microbial fermentation may result in the development of diarrhoea (Argenzio, 1978). An over production of microbial endproducts, *e. g.* due to dietary problems or secondary to carbohydrate malabsorption in the small intestine, produces luminal acidification and hyperosmolarity, and may lead to the development of osmotic diarrhoea (Argenzio, 1978, Holtug and Mortensen, 1989, Schmitt *et al.*, 1976). High faecal concentrations of SCFAs have been observed in diarrhoea associated with carbohydrate malabsorption (Schmitt *et al.*, 1976). An intra-rectal instillation of acetic acid in rats and cats resulted in the development of diffuse colitis similar to the human disease (McCafferty and Zeitlin, 1989). Butyric acid instillation in mice produced a reproducible, concentration-dependent colitis at concentrations between 1 and 12%, but this could not be reproduced by either low pH or by the administration of the butyrate anion at

neutral or alkaline pH (McCafferty and Zeitlin, 1989). Changes in SCFA production in patients with ulcerative colitis may be due to the bacterial fermentation of blood (Holtug *et al.*, 1988). *In vitro*, the addition of blood to a faecal incubation system resulted in a change in the SCFA production from acetate and propionate production to butyrate, isobutyrate, isovalerate and valerate domination (Holtug *et al.*, 1988). In man, increased SCFA (acetic, propionic and lactic) concentrations in non-strangulated intestinal obstruction have been related to the inhibition of water and electrolyte absorption, which leads to bowel distension (Fukushima *et al.*, 1983).

1.24 Mechanisms of antimicrobial alteration of gastrointestinal microflora

Antimicrobial-associated depression or alteration of the normal gastrointestinal microflora may be due to either an overgrowth of pathogens or to to inhibition of the normal microbial activity and VFA production resulting in digestive upsets, inappetence and a reduction in gastrointestinal motility (Argenzio, 1975, Prins, 1987).

It is worth considering the effects of antimicrobial agents on the ruminal microflora since these have been studied extensively. The effects of antimicrobial agents in the equine caecum are likely to be similar. Antimicrobial agents may alter ruminal bacterial fermentation following oral administration. In sheep, ruminal function was depressed following oral administration of penicillin G, streptomycin, chloramphenicol, terramycin and aureomycin (Prins, 1987). In addition, some antimicrobial agents alter ruminal fermentation following parenteral administration, due to excretion through the rumen wall or in saliva (Prins, 1987). Several antimicrobial agents, including penicillin G, ampicillin, methicillin, erythromycin, oleandomycin and oxytetracycline, are effective inhibitors of rumen cellulolysis *in vitro*, and can be assumed to damage the rumen ecosystem *in vivo*, whereas the aminoglycosides streptomycin, kanamycin and neomycin have no effect on cellulose breakdown (Prins, 1965, Wright, 1961). Penicillin G was inactivated rapidly in the rumen but despite this it reduced cellulolytic activity both *in vitro* and *in vivo* (Prins, 1987). In ruminal fluid *in vitro*, a penicillin/streptomycin combination reduced gas production, a measure of microbial activity, by a process that appeared to be dependent on the exposure time of the organisms to the antimicrobial agents (Klopfenstein *et al.*, 1964).

Defaunation decreases the proportions of acetate, butyrate and methane and increases the proportions of propionate in fermentation end-products in ovine ruminal fluid *in vitro* (Demeyer and Van Nevel, 1987). Antimicrobial agents used as feed additives (*e. g.* lasalocid, monensin, narasin, salinomycin and lysocellin) improve weight gain and feed conversion efficiency, reduce acetate, butyrate and methane production, reduce the ratio of acetate to propionate and increase propionate production (Bagley *et al.*, 1988, Chalupa *et al.*, 1980, Chirase *et al.*, 1988, Prins, 1987, Spears *et al.*, 1989). Chalupa *et al.* (1980) and

Sauer and Teather (1987) showed that the addition of monensin to rumen fluid *in vitro* inhibited methanogenesis, reduced the ratio of acetate to propionate, reduced acetate and butyrate production and increased valerate production. Amicloral reduces acetate production, increases ruminal propionate and butyrate production and almost completely inhibits methanogenesis (Chalupa *et al.*, 1980). *In vitro* all the antimicrobial agents tested (avoparcin, lasalocid, monensin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin, lonomycin, nigericin, cationomycin and lysocellin) increased the molar proportion of propionate (Hillaire *et al.*, 1989, Nagaraja *et al.*, 1987) and reduced the final pH and L-lactate concentrations in ruminal fluid (avoparcin, lasalocid, monensin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin) (Nagaraja *et al.*, 1987). Antimicrobial treatment did not affect the total VFA concentrations in ruminal fluid, except at high concentrations of tylosin and virginiamycin when there was a reduction in the total VFA concentration (Nagaraja *et al.*, 1987). In addition, there was an increase in the proportion of acetate at high concentrations (greater than 6 μg/ml) of tylosin, monensin, virginiamycin and thiopeptin, in ruminal fluid *in vitro* (Nagaraja *et al.*, 1987). Lasalocid, monensin and thiopeptin reduce lactate production and enhance lactate utilization in the rumen and are effective in treating ruminal lactic acidosis (Muir *et al.*, 1980, Nagaraja *et al.*, 1982). Nagaraja *et al.* (1987) reported that narasin and salinomycin were the most effective enhancers of propionate production in ruminal fluid *in vitro*, and that the ionophore compounds produced a greater inhibition of butyrate production than the non-ionophore compounds. In bovine ruminal fluid *in vitro*, administration of chloral hydrate, chloroform or triethanol reduced acetate, propionate and butyrate production and increased lactate production, and chloroform and chloral hydrate inhibited methane production (Amgarten *et al.*, 1981). The reduction in total VFA production appeared to be due to inhibition of the reductive pathway leading from lactate to propionate, resulting in an accumulation of lactate and a reduction in propionate concentrations.

1.25 The effects of the gastrointestinal tract on drug absorption

Drugs, chemicals, food substances and other factors may prevent an antimicrobial agent from reaching the systemic circulation following oral administration (Powers and Mercer, 1980). Chemical and physical binding and interactions of drugs occur in the gastrointestinal lumen and these alter the rate of transport or rate of metabolism of inactive compounds. This may result in an increase or a reduction in drug transport.

In man, under controlled conditions, there was a range of drug absorption following oral administration due to variations in gastrointestinal factors, *e. g.* gastric pH, rate of gastric emptying, gastrointestinal motility, transit time and the area of mucosa available for absorption (Macleod *et al.*, 1974). Following oral antimicrobial administration, the presence

of food in the gastrointestinal tract affects drug absorption profoundly, due to either chemical interactions between the ingesta and the drug, or physiological alterations in the intestinal tract due to the presence of food (Bogan *et al.*, 1984, Watson, 1986). Cellulose has a high capacity for drug binding and, in the horse feeding reduced the systemic availability of trimethoprim and phenylbutazone (Bogan *et al.*, 1984, Bogan and Marriner, 1987). In addition, the pH of the gastrointestinal tract is affected by feeding and is less acid when the digesta volume is largest. The presence of gastric ingesta influences the absorption of penicillin V in man and the dog (Ducharme *et al.*, 1983). In the equine, Wilson *et al.* (1985) noted that the absorption of cefadroxil monohydrate was poor and inconsistent. Ampicillin is absorbed poorly following oral administration to calves (Ziv and Horsey, 1979). Inefficient absorption and high concentrations of an antimicrobial agent remaining in the gastrointestinal lumen following oral administration may lead to disruption of the gastrointestinal microflora. However, the absorption of some agents is improved by the presence of food. The presence of bran strongly increased the intestinal absorption of chloramphenicol palmitate and it was suggested that the improved absorption may be due to more rapid intestinal hydrolysis of the drug by the increased activity of pancreatic lipase on bran (Buéno *et al.*, 1984).

The microorganisms in the rumen and the intestine may metabolize and inactivate certain antimicrobial agents. For example, chloramphenicol is absorbed very well following oral administration to dogs, cats, horses and pigs, but it has no therapeutic activity in the ruminant due to breakdown by ruminal microorganisms (Powers and Mercer, 1980). Virginiamycin is metabolized extensively, resulting in a reduction in antimicrobial activity, in bovine ruminal fluid *in vitro* (Gottschall *et al.*, 1988). In the horse, microbial activity in response to feeding could affect drug availability (Bogan *et al.*, 1984). In addition, bacterial fermentation in the caecum may release drug bound to cellulose, or a drug may be destroyed by microbial metabolism (Bogan *et al.*, 1984).

Aims

This project set out to examine the disposition of four antimicrobial agents in *Equidae*, with particular reference to the interactions with the gastrointestinal microflora. The plasma disposition and pharmacokinetics were studied following intravenous administration of penicillin G, ampicillin, amikacin and oxytetracycline to horses, ponies and donkeys and the caecal disposition was examined, in ponies with cannulated caecal fistulas, following intravenous and oral drug administration. Serial samples of caecal liquor or faecal material underwent bacteriological examinations to enumerate the commensal microflora and to detect the presence of pathogenic bacteria. Gastrointestinal fermentation was monitored, prior to and following antimicrobial administration, by measurement of caecal luminal pH and caecal and faecal SCFA concentrations. The presence or absence of diarrhoea was assessed by estimating faecal dry matter content and by observing and recording faecal consistency. The development of clinical signs, such as depression and anorexia, were recorded. Factors that may affect the systemic availability of an orally administered drug were studied *in vitro*. Antimicrobial agents were incubated in caecal contents in an anaerobic environment as a means of studying intra-caecal drug destruction or binding and *in vitro* alterations in SCFA concentrations. In addition, antimicrobial agents were incubated in buffer at gastric pH to determine the percentage of an agent remaining following passage through the stomach. *In vitro* studies of the binding of antimicrobial agents to hay at gastric and small intestinal pH were carried out.

2 Materials and Methods

2.1 Animals

Six thoroughbred horses (5 geldings and 1 mare) (numbers 1-6), aged 7-22 years, and weighing 489-613 kg, were used. Seven ponies (2 geldings and 5 mares) (numbers 7-13), aged 5-20 years and weighing 161-386 kg, were used. Four donkeys, (1 stallion and 3 mares) (numbers 14-17), aged 2-20 years, and weighing 161-213 kg, were used. In addition, 3 pony mares (numbers I-III), with caecal fistulas, aged 5-6 years and weighing 198-260 kg were used.

Management

All animals were housed in stable accommodation for at least 3 days prior to and during the experimental procedures. Ponies with caecal fistulas were housed at all times. Animals were fed hay twice daily (0900 h and 1600 h approximately), no concentrate feed was given. Water was available *ad libitum*.

2.2 Antimicrobial agents

Penicillin G (benzylpenicillin sodium BP, Crystapen 600 mg, Glaxo Laboratories Limited, Greenford, U. K.) dissolved in 4 ml water (sterile pyrogen-free water for injections, Animalcare Limited, York, U. K.) was used for both intravenous and oral administration.

Ampicillin (ampicillin sodium B. P. (Vet.), Penbritin™ veterinary injectable 2 g, Beecham Animal Health, Brentford, U. K.) was dissolved in 20 ml water for injections and was used for intravenous and oral administration.

Amikacin (amikacin sulphate, 250 mg/ml, Amiglyde-V, Fort Dodge Laboratories Inc, Iowa, U. S. A., Willows Francis Limited, Crawley, U. K.) was used for both intravenous and oral administration.

Oxytetracycline (oxytetracycline hydrochloride Ph Eur, 100 mg/ml, Terramycin* Q-100 injectable solution, Pfizer Limited, Sandwich, U. K.) was used for intravenous administration. Oxytetracycline (oxytetracycline hydrochloride, 200 g/kg, in a water soluble base, Terramycin* soluble powder concentrate 20%, Pfizer Limited, Sandwich, U. K.) dissolved in approximately 150 ml sterile water, was used for oral administration.

2.3 Antimicrobial agent administration

Intravenous drug administration

Slow (30-90 s depending on injection volume) intravenous injection was *via* a sterile needle (Microlance 25 mm long, 0.9 mm diameter, Becton-Dickinson, Dublin, Ireland) into a jugular vein. Prior to injection, some blood was drawn into the syringe containing the drug to check that the needle was lying in the lumen of the vein and not against the vessel wall. During injection, blood was drawn into the syringe 3-6 times (depending on injection volume) to check that the needle was still in a suitable position in the vein.

Penicillin G, ampicillin and oxytetracycline were administered at a dose rate of 10 mg/kg bwt. Amikacin was administered at a dose rate of 6 mg/kg bwt.

Oral drug administration

Oral drug administration was *via* a clean nasogastric tube (3 m long, 15 mm diameter, Vet Drug Company plc, Falkirk, U. K.). The nasogastric tube was warmed and softened by placing in a bucket of hot (40-50 °C) tap water for 5-10 min and the first 15 cm (approximately) of the end of the tube to be inserted into a nostril was lubricated (Lubrel, Arnolds Veterinary Products, Romford, U. K.). The lubricated end of the nasogastric tube was inserted into the medial aspect of the right nostril, taking care to avoid the false nostril, and pushed gently along the nasal passage with the animal's neck maintained in flexion. The progression of the tube was halted, in the region of the epiglottis, until swallowing occurred at which time the tube was fed gently down the oesophagus into the stomach. Prior to nasogastric intubation the tube was held on the outside of the animal to correspond with its direction of passage and the desired length was marked in indelible ink on the exterior of the nasogastric tube. Correct positioning of the tube was confirmed by blowing gently down the tube and listening for gurgling sounds, which indicated that tube was lying in the stomach. If nothing happened when the position of the nasogastric tube was checked then it was withdrawn slightly (5-10 cm) and the test repeated in case the tube had become kinked or the end blocked, *e. g.* by lying against the stomach wall. The drug solution was poured carefully into the rostral end of the tube and the tube end was held up at arm's length. The drug solution was followed by approximately 200 ml of cold tap water. Any fluid remaining in the tube was expelled into the stomach by gentle blowing. The tube was withdrawn slowly to allow any fluid remaining to trickle out into the oesophagus.

Penicillin G, ampicillin and oxytetracycline were administered at a dose rate of 10 mg/kg bwt. Amikacin was administered at a dose rate of 6 mg/kg bwt.

2.4 Caecal fistulation technique

Animals

One pony mare, aged 5 years and weighing 260 kg was fistulated, prior to the commencement of the present studies, using a procedure similar to the one outlined below, and was acquired with the caecal fistula *in situ*. Two pony mares aged 5 years and weighing 230 and 256 kg were selected for a two stage caecal fistulation procedure similar to that described by Boyd (1985). Prior to general anaesthesia, the animals were starved overnight (approximately 18 h) to reduce the volume of the intestinal contents. In addition, the hair was clipped from an area between the spine and ventral midline, and between the femur and the thirteenth rib.

Anaesthesia

Intravenous drug administration was *via* an intravenous cannula (Vygon Intraflon 2, 80 mm long, 2.1 mm diameter, Vet Drug Company plc, Falkirk, U. K.) placed in the right jugular vein. Prior to cannulation, the hair in the jugular groove region of the neck was clipped and the area cleaned. Local anaesthesia of the area was produced by subcutaneous injection of approximately 2 ml of lignocaine hydrochloride solution (30 mg/ml, Lignocaine-A Injection, Univet Limited, Bicester, U. K.) into the area where the cannula was to be inserted. A small skin incision (approximately 5 mm) was made with a sterile scalpel blade, and the intravenous cannula was inserted through the skin incision into the jugular vein, and a three-way tap (Vet Drug Company plc, Falkirk, U. K.) attached in the closed position. Prior to insertion, the cannula and three-way tap were flushed with a small volume (3-5 ml) of heparinized saline (4 units per ml, 0.25 ml heparin sodium injection BP (mucous) (1000 units per ml, Evans, Langhurst, U. K.) diluted in 500 ml of sterile normal saline (Aquapharm 0.9% w/v sodium chloride intravenous infusion BP, Animalcare Limited, York, U. K.). The cannula and three-way tap were held in position by suturing to the skin of the neck.

In each instance, the animal was premedicated by intravenous administration of xylazine (20 mg/ml, Rompun, Bayer UK Limited, Bury St Edmunds, U. K.) at a dose rate of 1.1 mg/kg bwt. Two minutes after xylazine administration, general anaesthesia was induced by intravenous administration of ketamine hydrochloride (100 mg/ml, Vetalar, Parke, Davis and Company, Pontypool, U. K.) at a dose rate of 2.2 mg/kg bwt. After induction of general anaesthesia, the animal was intubated with an endotracheal tube (18 or 20 mm diameter) and general anaesthesia was maintained using 2-4% halothane (Fluothane*,

Coopers Pitman-Moore, Crewe, U. K.) in oxygen (BOC, Glasgow, U. K.) at a flow rate of 4-6 l/min.

Postoperative analgesia was maintained by intramuscular administration of pethidine hydrochloride (50 mg/ml, Arnolds Veterinary Products, Romford, U. K.) at a dose rate of 2 mg/kg bwt, intravenous administration of flunixin meglumine (Finadyne* solution, 50 mg/ml, Schering-Plough Animal Health, Mildenhall, U. K.) at a dose rate of 1.1 mg/kg bwt or intravenous administration of butorphanol tartrate (Torbugesic, 10 mg/ml, C-Vet Limited, Bury St Edmunds, U. K.) at a dose rate of 1.1 mg/kg bwt.

Cannula design

The cannula was based on the design described by Simmons and Ford (1988). Unlike their design the cannula was 'T' shaped and split in two lengthwise, to allow the insertion of the 2 'L' shaped halves (a semicylindrical limb (80 mm long, 25 mm diameter, 15 mm id) angled at approximately 120 ° to the threaded cannula stem (120 mm long, 25 mm diameter, 15 mm id)) through a small incision in the caecal wall. The cannula was held in place by two rings (80 mm diameter with 25 mm diameter hole in centre) which were screwed onto the threaded stem of the cannula until secure against the flank of the animal. The flank was protected by a piece of rubber which was cut to the same size and shape as the securing rings. The cannula and rings were made out of solid plastic. The cannula design is shown in Figure 2-1.

Caecal fistulation

After induction of general anaesthesia, the animal was placed in left lateral recumbency with its hind limbs supported and tied in extension. After suitable preoperative preparation, a vertical incision, approximately 20 cm long, was made in the right flank midway between the spine and ventral midline and between the last rib and the ipsilateral hind limb. The caecum was localised within the abdomen by palpation of the characteristic four longitudinal muscular bands (Nickel *et al.*, 1979) and was orientated so that part of the caecal wall could be sutured onto the abdominal wall. The caecum was attached to the abdominal musculature by a double ring (approximately 3 cm diameter) of non-absorbable sutures (simple interrupted pattern, Prolene 1, Vet Drug Company plc, Falkirk, U. K.). A similar pattern of absorbable sutures (Vicryl 0, Vet Drug Company plc, Falkirk, U. K.) was used to appose the muscular layers of the dorsal $^1/_2$-$^2/_3$ of the incision. The dorsal $^1/_2$-$^2/_3$ of the skin incision was closed using non-absorbable sutures (Prolene 1, Vet Drug Company plc) in a simple interrupted pattern. The animal was allowed to recover from the anaesthetic and the flank wound was allowed to heal. The flank wound was bathed with sterile normal saline (Aquapharm 0.9% w/v sodium chloride intravenous infusion BP, Animalcare Limited,

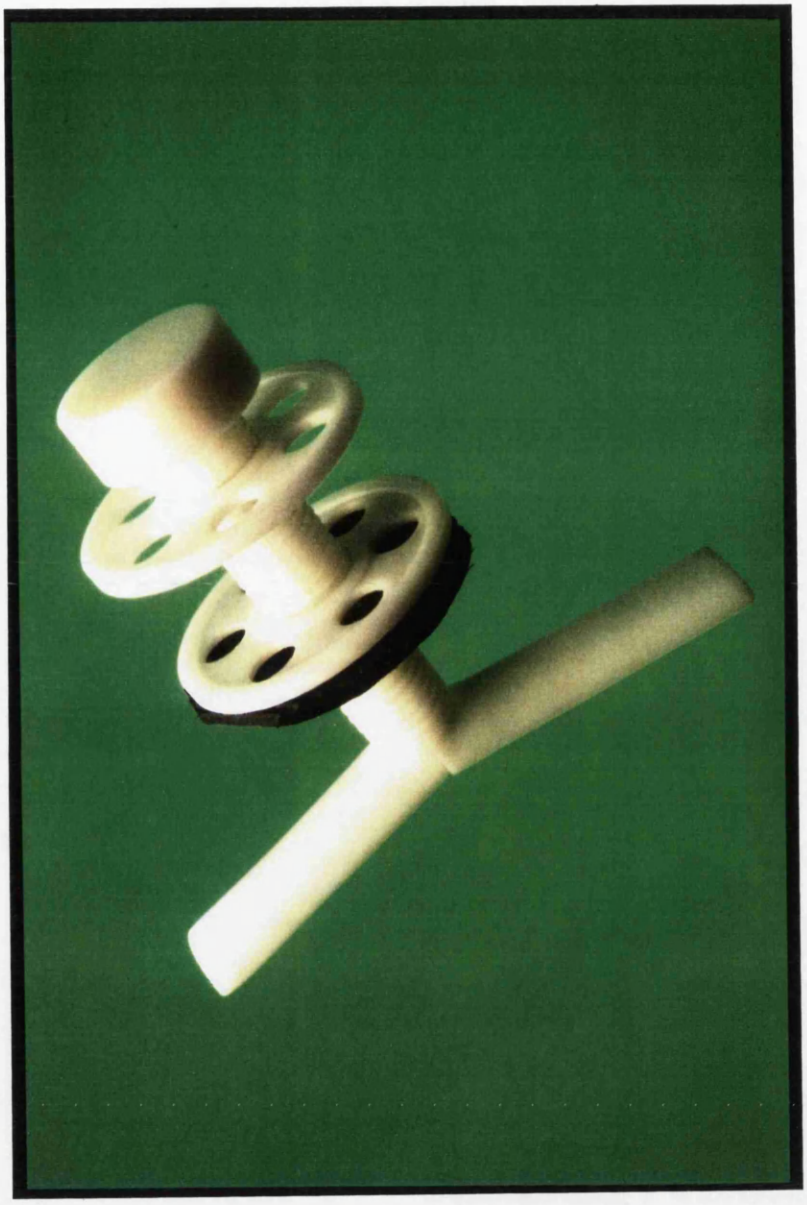

Figure 2-1. Caecal cannula design

York, U. K.), and the skin stitches were removed after 7-10 days. Antimicrobial therapy was maintained for 7 days with ampicillin sodium (Penbritin, Beecham Animal Health, Tadworth, U. K.) at a dose rate of 10 mg/kg bwt every 12 h. Figure 2-2a shows an animal 10 days after the first stage of the caecal fistulation procedure.

The second stage was carried out 4-6 weeks after the first stage. The animal was anaesthetized, and placed in left lateral recumbency. After suitable pre-operative preparation, approximately 5 cm of the ventral aspect of the original incision site was incised carefully until the caecal wall was located. A small (2-3 cm) 'X' shaped incision was made in the caecal wall and the 2 'L' shaped halves of the cannula inserted. The 2 halves of the cannula were held together and the end of the resultant cannula was plugged with a swab to prevent leakage of the caecal contents. The cannula was put under slight lateral tension and sutures (where required) were put in to appose the exposed abdominal muscle and skin layers. The rubber protective ring and the 2 plastic rings were screwed onto the cannula stem to hold it in place loosely against the animal's flank, the swab was removed, and the cap was screwed on. The animal was allowed to recover from the anaesthetic and the flank wound was allowed to heal. Post-operative care was the same as in the first stage of the procedure. On a daily basis, the flank skin proximal to the cannula was bathed to remove debris and was protected from damage, caused by the leakage of caecal fluid, by the liberal application of white soft paraffin BP (Vet Drug Company plc, Falkirk, U. K.) until healing was complete.

The flank wound healed by granulation until there was little or no leakage around the cannula. Once healing was complete, the area around the cannula was kept clean by washing with tap water, when required. Hair was clipped from the flank in the region of the cannula when necessary. The cannula was left in place for 4-6 weeks prior to the start of experimental procedures. Figure 2-2b shows an animal 8 weeks after the second stage of the caecal fistulation procedure.

2.5 Sampling techniques and sample preparation

Blood Sampling Technique

Blood, for drug analysis and plasma biochemistry, was collected from a jugular vein into 10 ml lithium heparin syringes (Li-Heparin LH/10 Monovette®, Sarstedt Limited, Loughborough, U. K.) using a sterile needle (Microlance, 25 mm long, 0.9 mm diameter, Becton-Dickinson, Dublin, Ireland). Blood, for haematological examinations, was collected in a similar manner into 5 ml potassium ethylenediaminetetracetic acid syringes (K-EDTA, Monovette®, Sarstedt Limited).

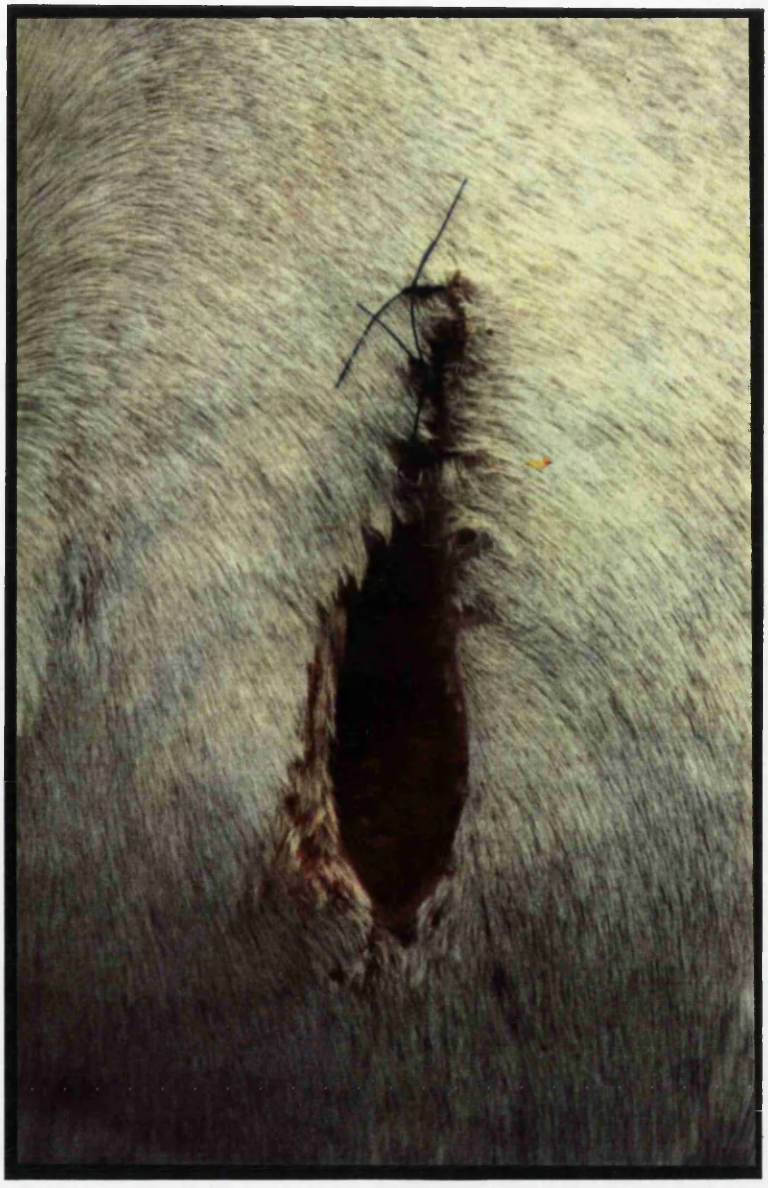

Figure 2-2a. The flank of an animal 10 days after the first stage of the caecal fistulation procedure

Figure 2-2b. The flank of an animal 8 weeks after the second stage of the caecal fistulation procedure

Intestinal fluid sampling technique

Samples of caecal liquor were taken by removing the cap from a caecal cannula. The cannula was cleared and an aliquot of caecal liquor was removed using a piece of clean polythene tubing of approximately 1 cm in diameter. Caecal liquor was collected into a clean glass universal container with a screw top.

Faecal sampling technique

Faecal samples were taken from the rectum using adequate lubricant gel (Lubrel, Arnolds Veterinary Products, Romford, U. K.) applied to a gloved (Nublu Arm-length disposable gloves, Arnolds Veterinary Products, Romford, U. K.) hand. The samples were placed directly into faecal cartons with lids (Bettercater Limited, Edinburgh, U. K.).

Sample Preparation

After collection, blood samples in lithium heparin tubes were centrifuged at 1800 *g* for 10 min in a cooled (5-15 °C) centrifuge (MSE Chilspin, M. S. E. Scientific Instruments, Crawley, U. K.). Plasma was decanted into 10 ml neutral tubes (Sarstedt Limited, Loughborough, U. K.) and stored at either 4 °C overnight until analysed by bioassay for penicillin G, ampicillin, amikacin or oxytetracycline, or at -20 °C until analysed by HPLC for oxytetracycline. The samples for plasma biochemistry were submitted for analysis on the day of collection. The samples for haematological examination were mixed well and submitted for analysis on the day of collection. Caecal liquor samples were decanted into 10 ml neutral tubes and stored at 4 °C until drug analysis, and then at -20 °C until analysis for SCFA content. Faecal samples were stored in faecal cartons in a similar manner to caecal liquor.

The samples for the bioassay were analysed as soon as possible after the experiments took place (within approximately 2 days). Standard plasma, caecal liquor or faecal samples were made on the day of the experiment and stored along with the unknown samples. Other analyses were carried out as soon as possible (within 14 days of sampling where possible).

2.6 General solutions

All water, unless stated otherwise, was distilled in the presence of potassium permanganate.

Hydrochloric acid solution (0.2 M)

The solution was made from 20 ml of a stock solution of 1 M hydrochloric acid (50 ml 10 M hydrochloric acid (Convol®, The British Drug House Chemicals Limited) in 500 ml water) diluted to 100 ml with water.

Potassium dihydrogen orthophosphate solution (0.2 M)

The solution was made from 27.22 g potassium dihydrogen orthophosphate (FW 136.09 g, The British Drug House Chemicals Limited, Poole, U. K.) dissolved in 1 l water.

Sodium hydroxide solution (0.2 M)

The solution was made from 8 g of sodium hydroxide (FW 40 g, pellets pure analytical reagent, Koch-Light Limited, Haverhill, U. K.) dissolved in 1 l water.

Sodium hydroxide solution (1 M)

The solution was made from 40 g of sodium hydroxide dissolved in 1 l water.

Potassium hydroxide solution (10 M)

The solution was made from 56.11 g (FW 56.11 g, Analar®, The British Drug House Chemicals Limited) dissolved in 100 ml water.

Potassium chloride solution (0.2 M)

The solution was made from 14.91 g potassium chloride (FW 74.55 g, The British Drug House Chemicals Limited) dissolved in 1 l water.

Phosphate buffer (0.05 M, pH 7.0)

Stock phosphate buffer solution (0.05 M, pH 7.0) was made from 250 ml of 0.2 M potassium dihydrogen orthophosphate and 148 ml of 0.2 M sodium hydroxide, made up to 1 l with water.

Phosphate buffer (0.05 M, pH 8.0)

Stock phosphate buffer solution (0.05 M, pH 8.0) was made from 250 ml of 0.2 M potassium dihydrogen orthophosphate and 234 ml of 0.2 M sodium hydroxide, made up to 1 l with water.

Phosphate buffer (0.05 M, pH 4.5)

Stock phosphate buffer solution (0.05 M, pH 4.5) was made from 250 ml of 0.2 M potassium dihydrogen orthophosphate, made up to 1 l with water and with the pH adjusted using 10 M potassium hydroxide.

Chloride buffer (0.02 M, pH 1.9)

Stock chloride buffer solution (0.02 M, pH 1.9) was made from 50 ml 0.2 M potassium chloride and 50 ml 0.2 M hydrochloric acid diluted in 1 l with water.

The pH of the buffer solutions was checked using a digital pH/temperature meter (Fisons Scientific Equipment, Loughborough, U. K.). The pH meter was calibrated to pH 7.0 using pH 4.0 and pH 7.0 buffer (±0.01 at 20°C, The British Drug House Chemicals Limited, Poole, U. K.). The pH meter was calibrated between measurements, after washing with deionized water (Ionmiser, Model 2C, Houseman Hegro, Slough, U. K.) and blotting dry.

2.7 Measurement of antibiotics by agar gel diffusion assay

A bioassay similar to that described by Bennett *et al.* (1966) was used to measure penicillin G, amikacin, ampicillin and oxytetracycline in equine plasma, caecal liquor and faeces.

Spore preparation

Vials of ATCC spore cultures (1 ml per vial, Bacto®, Difco Laboratories Limited, East Molesey, U. K.) were stored at 4 °C until required. The contents of a vial of spore culture were diluted in approximately 19 ml of sterile normal saline (0.9% w/v sodium chloride dissolved in water) in a glass universal bottle. The resultant diluted spore suspension was stored at 4 °C until required.

Preparation of sterile growth medium

Agar, of a suitable pH for the antibiotic assay and for the growth of the chosen test organism, was dissolved in hot tap water at the recommended concentration in a 2 l glass conical flask. The mixture was stirred with a glass stirring rod, the top of the flask covered with aluminium foil to prevent excessive evaporation and the flask placed in a steamer (100 °C, Laboratory Thermal Equipment, Oldham, U. K.) for 90 min or until the mixture was clear, and the contents stirred occasionally. The agar was divided into aliquots of approximately 310 ml using a glass 500 ml measuring cylinder, and poured into 500 ml clean glass screw cap bottles. The bottles were capped loosely and sterilized in an autoclave (Autoclave 290EH, Harvard Apparatus and Instrument Services, Oldham, U. K.) for 15

min at 121 °C and 825 mm Hg, allowed to cool, the caps tightened, and stored at 4 °C until required.

Test of spore suspension concentration

Test plates were used to assess the concentration of the spore suspension which was needed to obtain a suitable colony density after incubation. A known volume of diluted spore suspension was added to each of six aliquots of the desired growth medium (Table 2-1). The resultant seeded agar was poured immediately into clean plastic petri dishes (90 mm diameter, triple vent, Greiner Labortechnik Limited, Dursley, U. K.), the lids replaced and the agar allowed to set (around 30 min at room temperature (20 °C)). Once the agar had cooled and solidified, a single 9 mm well was made in the centre of each plate and filled with a suitable standard solution.

There was no growth of *Bacillus spp.* at 0% spore suspension showing that no bacterial contamination of sterile agar or pipette tips had occurred. At 0.05% and 0.1% spore suspension, separate colonies were evident after incubation, and a zone of inhibition was present around the standard filled well but the edge of the zone was indistinct. The most satisfactory percentage of diluted spore suspension was 0.33% (*i. e.* 1 ml diluted spore suspension per 300 ml agar). This concentration of diluted spore suspension produced a confluent lawn of colonies whilst still leaving a clear, well defined zone of inhibition around the well containing the antibiotic standard solution. At 0.5 and 1.0% spore suspension the growth of the bacterial lawn was thicker and, although the inhibition zone was defined clearly, there was some reduction in the diameter of the inhibition zone.

Stock sterile buffer solutions

Penicillin G and ampicillin were dissolved in phosphate buffer (0.05 M, pH 7.0). Amikacin was dissolved in phosphate buffer (0.05 M, pH 8.0). Oxytetracycline was dissolved in phosphate buffer (0.05 M, pH 4.5). Buffer was divided into aliquots of approximately 500 ml, poured into clean 500 ml glass screw cap bottles, capped loosely, and sterilized in an autoclave for 15 min at 121 °C and 825 mm Hg (Autoclave 290EH, Harvard Apparatus and Instrument Services, Oldham, U. K.). After sterilizing, the screw caps were tightened and the buffer stored at 4 °C until required.

Standard solutions

On the day of assay, a 200 µg/ml standard solution was made by weighing 0.02 g of standard compound and dissolving it in a suitable sterile stock buffer solution, in a clean 100 ml glass volumetric flask, to a produce a final volume of 100 ml. A 20 µg/ml standard was

% diluted spore suspension	Agar (ml)	Volume diluted spore suspension (µl)
0	20	0
0.05	20	10
0.1	20	20
0.33	20	66
0.5	20	100
1.0	20	200

Table 2-1. Test of diluted spore suspension concentration used for agar gel diffusion assay

Plasma/caecal liquor concentration (µg/ml)	Stock standard solution (volume and concentration)	Plasma or caecal liquor volume (µl) (or 1 g faeces)
0	0	1
0.25	12.5 µl of 20 µg/ml	987.5
0.5	25 µl of 20 µg/ml	975
1	50 µl of 20 µg/ml	950
2	100 µl of 20 µg/ml	900
4	20 µl of 200 µg/ml	980
5	25 µl of 200 µg/ml	975
8	40 µl of 200 µg/ml	960
10	50 µl of 200 µg/ml	950
20	100 µl of 200 µg/ml	900
25	125 µl of 200 µg/ml	875
40	200 µl of 200 µg/ml	800
50	250 µl of 200 µg/ml	750
80	400 µl of 200 µg/ml	600
100	500 µl of 200 µg/ml	500

Table 2-2. Standard plasma, caecal liquor and faecal concentrations of antimicrobial drugs for agar gel diffusion assay

made by taking 10 ml, by glass bulb pipette, of the 200 µg/ml standard and diluting it to 100 ml with a suitable volume of sterile stock buffer solution in a clean glass volumetric flask.

Standards for the assay of unknown equine plasma and equine caecal liquor samples were prepared in drug-free equine plasma or drug-free equine caecal liquor using the 200 and 20 µg/ml stock standards described above. A range of plasma or caecal liquor standards was constructed to encompass the range of likely plasma or caecal liquor concentrations (Table 2-2). Plasma or caecal liquor was measured into 10 ml clean neutral tubes (Sarstedt Limited, Loughborough, U. K.), the required volumes of stock standard solution was added to each tube, a plastic push-in stopper (Sarstedt Limited) put in each tube and the plasma or caecal liquor and stock standard solutions mixed for approximately 10 s using a Griffin vortex shaker/stirrer (Griffin and George Limited, Loughborough, U. K.). Standards for assay of equine faecal samples were prepared in drug-free equine faeces using the 200 and 20 µg/ml stock standards. A range of faecal standards were constructed to encompass the range of likely faecal concentrations (Table 2-2). Faecal material was weighed into aliquots of 1 g and put into 10 ml clean neutral tubes. The required volume of stock standard solution was added to each tube and 3 ml of sterile phosphate buffer (0.05 M, pH 7.0) was added to each sample and a plastic push-in stopper put into each tube. The samples were then mixed using a Griffin vortex shaker/stirrer for 30 s and centrifuged for 15 min at 1800 *g*. The supernatant from each sample was put into the appropriate wells on agar plates.

Preparation of assay plates

The plates for the bioassays consisted of a 30 cm x 30 cm aluminium frame, an aluminium lid, a glass plate and 8 spring clips. Clean, dry, cool glass plates were held onto the aluminium frames using the spring clips, sterilized by flaming with a Bunsen burner and placed on a pre-levelled triangular levelling stand until the agar was poured.

On the day of an analysis the required number of bottles of agar were placed in a water bath (Laboratory Thermal Equipment, Oldham, U. K.) at 54 °C until warm (around 1 h) and placed in a steamer until liquid (around 1 h) before being allowed to cool. Once the liquid agar had cooled to a suitable temperature (around 1 h in a water bath at 54 °C), the required amount of diluted spore suspension (1 ml per 300 ml agar) was added. The seeded agar was mixed using a gentle swirling motion, *i. e.* sufficient to mix in the diluted spore suspension whilst avoiding air bubble formation, and poured onto the prepared plates in a circular pattern. The seeded agar was poured rapidly to avoid too much cooling, and gently enough to avoid spilling, and to cover the plate evenly. Any air bubbles formed during the mixing/pouring process were removed by piercing with a sterile needle. The lid of the plate

was replaced as soon as possible. The plates were left at room temperature (20 °C) until the agar had cooled and solidified (approximately 45 min).

Holes were punched in the solidified agar using a 9 mm diameter circular metal punch (C. P. Instrument Company Limited, Bishops' Stortford, U. K.) in a quasi latin square pattern. A 64 well plate (8 rows of 8 wells) design was used and this was arranged so that each sample only appeared once in each row or column. There were four replicates of each sample. This meant that samples were spread randomly over the area of the plate thus allowing for any variation in agar depth, pH or temperature. In general, there were 6 standard concentrations (*i. e.* 4 replicates of 6 standard concentrations), and 4 replicates of 10 samples of unknown concentration, per plate. A cardboard template, marked with the well pattern for the quasi latin square design, was placed directly under the plate. The loosened agar was removed using the blunt end of a disposable Pasteur micropipette (Bilbate Limited, Daventry, U. K.) attached by rubber tubing to a vacuum pump (Millipore U. K., Watford, U. K.). Waste agar was collected in a conical flask placed between the disposable Pasteur micropipette and the vacuum pump.

Assay preparation and incubation

A known volume (170 µl) of each standard or sample solution was measured into 4 wells using the cardboard quasi latin square template as a guide. A fresh pipette tip was used for each standard or sample. Once all the wells on a plate were filled, the lid of the plate was replaced gently and the plate placed carefully in an incubator at 30 °C (Gallenkamp Size 3 Economy incubator with fan, Fisons Scientific Equipment, Loughborough, U. K.) overnight (18-21 h).

Reading plates

The plates were removed from the incubator, the lids removed and the plates placed on the quasi latin square template. The zones of inhibition were measured using an electronic digital calliper (Fowler and NSK Max-cal, C. P. Instrument Company Limited, Bishops' Stortford, U. K.) and recorded using a printer (Fowler QC EDP-100, C. P. Instrument Company Limited). After reading and recording the 4 zone diameters for each sample, the statistics facility on the printer was used to calculate and record the arithmetic mean, maximum, minimum, range and SD of each sample.

Calculation of results

A programmable calculator (TI Programmable 59, Texas Instruments, Texas, U. S. A.) was used to calculate the concentration of each unknown sample from the mean zone diameter of that sample. The zone diameter (mm) was related to the $\log_{10}$ of the standard

concentrations. By entering the $\log_{10}$ of the concentration and the mean zone diameter (mm) for each standard concentration, a regression line (line of best fit for $\log_{10}$ concentration versus zone diameter plot) and r was calculated for each plate. The $\log_{10}$ of the concentration of an unknown sample was calculated from the regression line, from the relevant plate, using the mean zone diameter of that sample, and the concentration was calculated by taking the antilogarithm of this number (2 decimal places).

Precision

The precision of each agar gel diffusion assay was assessed using the coefficients of variation of the mean zone diameter (mm) of each replicate plate (4 wells per plate), that is the sample SD expressed as a percentage of sample mean, of equine plasma, caecal liquor or faeces standard samples to which antimicrobial preparation had been added and analysed in a single assay (within-day or intra-assay variation) and on different days (between-day or inter-assay variation).

Linearity and Sensitivity

A computer programme (Cricket graph version 1.3, Cricket software, Pennsylvania, U. S. A.) was used to plot a graph of zone diameter (mm) (mean±SD) versus $\log_{10}$ concentration (μg/ml) and to calculate the line of best fit, r and the limit of detection for the replicate plates of standard equine plasma, caecal liquor and faecal concentrations used for the method validation. The sensitivity of each assay was calculated, from the equation of the line of best fit, using a minimum zone diameter of 10 mm.

2.8 Agar gel diffusion assay of penicillin G sodium

A spore suspension of *Bacillus subtilis* (ATCC 6633) was used as the test organism. The test agar used was antibiotic medium 2 (pH 6.6, Bacto®, Difco Laboratories Limited, East Molesey, U. K.) at a concentration of 2.55% w/v agar base dissolved in water. Penicillin G (benzylpenicillin G sodium salt, Sigma Chemical Company Limited, Poole, U. K.) for standard solutions was dissolved in stock sterile phosphate buffer solution (0.05 M, pH 7.0). Plasma, caecal liquor or faecal standards were made on the day of the experiment.

Precision

The precision of the agar gel diffusion assay of penicillin G sodium in equine plasma was good as shown by the low (<6%) values of the within-day and between-day coefficients of variation of the zone diameter (mean±SD) of 3 plates analysed in a single assay and 8 plates analysed on different days (Table 2-3).

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%) Within-day	Between-day
0	0.00±0.00 (n=11)	0.00 (n=3)	0.00 (n=8)
0.25	23.36±1.22 (n=11)	1.70 (n=3)	5.94 (n=8)
1	30.17±0.96 (n=11)	1.79 (n=3)	3.51 (n=8)
5	36.55±1.05 (n=11)	1.81 (n=3)	3.06 (n=8)
10	39.03±1.24 (n=11)	2.06 (n=3)	3.55 (n=8)
20	41.01±1.03 (n=11)	2.30 (n=3)	2.54 (n=8)
40	43.84±0.71 (n=11)	1.33 (n=3)	1.73 (n=8)
80	46.58±0.95 (n=11)	0.42 (n=3)	2.36 (n=8)

Table 2-3. Zone diameters and coefficients of variation of penicillin G sodium in equine plasma

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%) Within-day	Between-day
0	0.00±0.00 (n=11)	0.00 (n=3)	0.00 (n=8)
0.25	22.96±1.06 (n=11)	5.68 (n=3)	4.24 (n=8)
1	30.40±1.18 (n=11)	3.42(n=3)	3.65 (n=8)
5	36.52±0.71 (n=11)	1.91 (n=3)	1.96 (n=8)
10	39.46±0.98 (n=11)	0.92 (n=3)	2.68 (n=8)
20	42.13±0.98 (n=11)	1.36 (n=3)	2.48 (n=8)
40	44.24±1.03 (n=8)	NS	2.34 (n=8)
80	45.88±1.39 (n=8)	NS	3.03 (n=8)

Table 2-4. Zone diameters and coefficients of variation of penicillin G sodium in equine caecal liquor

The precision of the agar gel diffusion assay of penicillin G sodium in equine caecal liquor was good as shown by the low (<6%) values of the within-day and between-day coefficients of variation of the zone diameter (mean±SD) of 3 plates analysed in a single assay and 8 plates analysed on different days (Table 2-4).

The precision of the agar gel diffusion assay for penicillin G sodium in equine faeces was good as shown by the reasonably low (<13%) values of the between-day coefficients of variation of the zone diameter (mean±SD) of 5 plates analysed on different days (Table 2-5).

Linearity and Sensitivity

The linearity of the zone diameters of penicillin G sodium was evaluated from the 11 replicate plates of standard equine plasma samples and was found to be good (r = 0.996) over the concentration range studied (0.25-80 μg/ml) (Figure 2-3). The overall limit of detection, 0.01 μg/ml, was calculated from the equation of the line of best-fit, $y = 29.59 + 9.05(\log_{10}x)$.

The linearity of the zone diameters of penicillin G sodium was evaluated from the 11 replicate plates of standard equine caecal liquor samples and this was found to be good (r = 0.989) over the concentration range studied (0.25-80 μg/ml)) (Figure 2-3). The overall limit of detection, 0.01 μg/ml, was calculated from the equation of the line of best-fit, $y = 29.64 + 9.16(\log_{10}x)$.

The linearity of the zone diameters of penicillin G sodium was evaluated from the 5 replicate plates of standard equine faecal samples and this was found to be good (r = 0.994) over the concentration range studied (0.25-10 μg/ml)) (Figure 2-3). The overall limit of detection, 0.07 μg/ml, was calculated from the equation of the line of best-fit, $y = 23.81 + 11.83(\log_{10}x)$.

2.9 Agar gel diffusion assay of ampicillin sodium

The agar and test organism used for the bioassay of ampicillin sodium were the same as described for penicillin G. Ampicillin (ampicillin sodium salt, Sigma Chemical Company Limited, Poole, U. K.) for standard solutions was dissolved in sterile phosphate buffer (0.05 M, pH 7.0). Plasma, caecal liquor or faecal standards were made on the day of the experiment and stored with the unknown samples at 4 °C.

Precision

The precision of the agar gel diffusion assay of ampicillin sodium in equine plasma was good as shown by the low (<8%) values of the within-day and between-day coefficients of

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of variation (%) Between-day
0	0.00±0.00 (n=5)	0.00 (n=5)
0.25	16.12±2.04 (n=2)	12.64 (n=2)
1	24.74±1.59 (n=5)	6.41 (n=5)
5	32.04±2.52 (n=5)	7.87 (n=5)
10	35.33±1.85 (n=5)	5.22 (n=5)

Table 2-5. Zone diameters and coefficients of variation of penicillin G sodium G in equine faeces

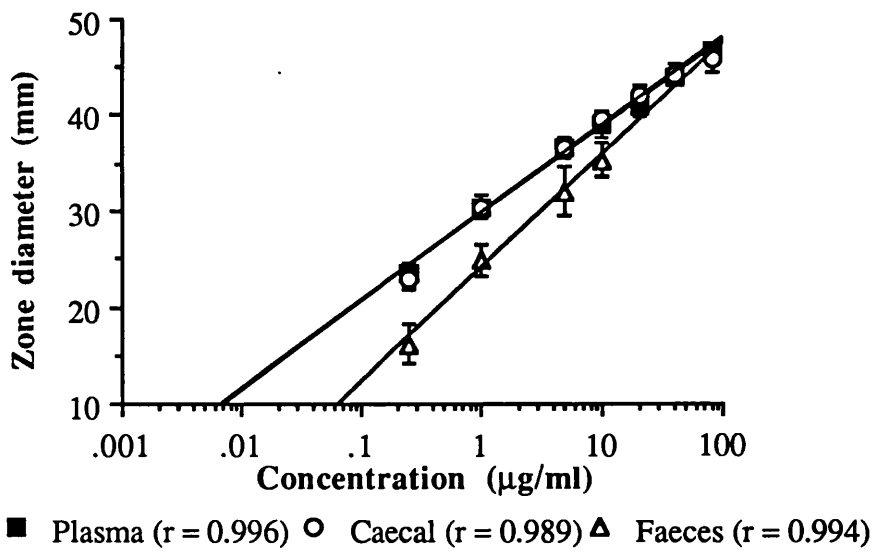

Figure 2-3. Zone diameters (mean±SD) of penicillin G sodium in equine plasma, caecal liquor and faeces

variation of the zone diameter (mean±SD) of 6 plates analysed in a single assay and 8 plates analysed on different days (Table 2-6).

The precision of the agar gel diffusion assay of ampicillin sodium in equine caecal liquor was good as shown by the low (<10%) values of the within-day and between-day coefficients of variation of the zone diameter (mean±SD) of 3 plates analysed in a single assay and 8 plates analysed on different days (Table 2-7).

The precision of the agar gel diffusion assay of ampicillin sodium in equine faeces was good as shown by the low (<9%) values of between-day coefficients of variation of the zone diameter (mean±SD) of 3 plates analysed on different days (Table 2-8).

The precision of the agar gel diffusion assay of ampicillin sodium in phosphate buffer (0.05 M, pH 7.0) was good as shown by the low (<8%) values of between-day coefficients of variation of the zone diameter (mean±SD) of 12 plates analysed on different days (Table 2-9).

Linearity and Sensitivity

The linearity of the zone diameters of ampicillin sodium was evaluated from the 14 replicate plates of standard equine plasma samples and this was found to be good ($r = 0.992$) over the concentration range studied (0.25-80 μg/ml) (Figure 2-4). The overall limit of detection, 0.02 μg/ml, was calculated from the equation of the line of best-fit, $y = 25.88 + 9.01(\log_{10}x)$.

The linearity of the zone diameters of ampicillin sodium was evaluated from the 11 replicate plates of standard equine caecal liquor samples and this was found to be good ($r = 0.980$) over the concentration range studied (0.25-80 μg/ml) (Figure 2-4). The overall limit of detection, 0.12 μg/ml, was calculated from the equation of the line of best-fit, $y = 20.92 + 11.89(\log_{10}x)$.

The linearity of the zone diameters of ampicillin sodium was evaluated from the 3 replicate plates of standard equine faecal samples and this was found to be good ($r = 0.993$) over the concentration range studied (0.5-20 μg/ml) (Figure 2-4). The overall limit of detection, 0.31 μg/ml, was calculated from the equation of the line of best-fit, $y = 16.10 + 12.17(\log_{10}x)$.

The linearity of zone diameters of ampicillin sodium was evaluated from the 12 replicate plates of standard phosphate buffer (0.05 M, pH 7.0) samples and this was found to be good ($r = 0.987$) over the concentration range studied (0.25-100 μg/ml) (Figure 2-4). The

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=14)	0.00 (n=6)	0.00 (n=8)
0.5	22.22±1.31 (n=11)	2.99 (n=6)	7.10 (n=5)
2	29.31±1.69 (n=11)	2.84 (n=6)	7.28 (n=5)
5	32.49±1.87 (n=14)	1.81 (n=6)	6.93 (n=8)
10	35.51±1.77 (n=14)	1.53 (n=6)	5.94 (n=8)
20	37.75±1.78 (n=14)	1.44 (n=6)	5.71 (n=8)
40	40.06±1.64 (n=14)	1.97 (n=6)	4.59 (n=8)
80	42.44±1.82 (n=11)	2.10 (n=6)	5.07 (n=5)

Table 2-6. Zone diameters and coefficients of variation of ampicillin sodium in equine plasma

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=11)	0.00 (n=3)	0.00 (n=8)
0.5	15.57±1.31 (n=7)	5.62 (n=3)	9.04 (n=4)
1	21.10±1.20 (n=11)	3.13 (n=3)	5.36 (n=8)
5	31.29±1.98 (n=11)	4.37 (n=3)	6.95 (n=8)
10	33.81±1.60 (n=11)	3.15 (n=3)	5.24 (n=8)
20	36.93±1.77 (n=11)	3.47 (n=3)	5.24 (n=8)
40	39.40±0.94 (n=4)	NS	2.38 (n=4)
80	42.07±1.07 (n=7)	2.18 (n=3)	2.61 (n=4)

Table 2-7. Zone diameters and coefficients of variation of ampicillin sodium in equine caecal liquor

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of variation (%) Between-day
0	0.00±0.00 (n=3)	0.00 (n=3)
0.5	11.95±0.97 (n=3)	8.15 (n=3)
1	16.42±1.20 (n=3)	7.34 (n=3)
5	24.77±1.31 (n=3)	5.30 (n=3)
10	29.10±1.37 (n=3)	4.71 (n=3)
20	31.09±0.96 (n=3)	3.09 (n=3)

Table 2-8. Zone diameters and coefficients of variation of ampicillin sodium in equine faeces

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%) Between-day
0	0.00±0.00 (n=12)	0.00 (n=12)
0.5	18.38±1.44 (n=4)	7.82 (n=4)
2	25.60±0.75 (n=4)	2.93 (n=4)
5	28.76±1.06 (n=4)	3.67 (n=4)
10	32.90±1.74 (n=9)	5.30 (n=9)
20	33.57±0.81 (n=4)	2.40 (n=4)
25	37.09±1.60 (n=8)	4.31 (n=8)
50	39.58±0.27 (n=8)	3.22 (n=8)
100	41.59±1.36 (n=8)	3.26 (n=8)

Table 2-9. Zone diameters and coefficients of variation of ampicillin sodium in phosphate buffer

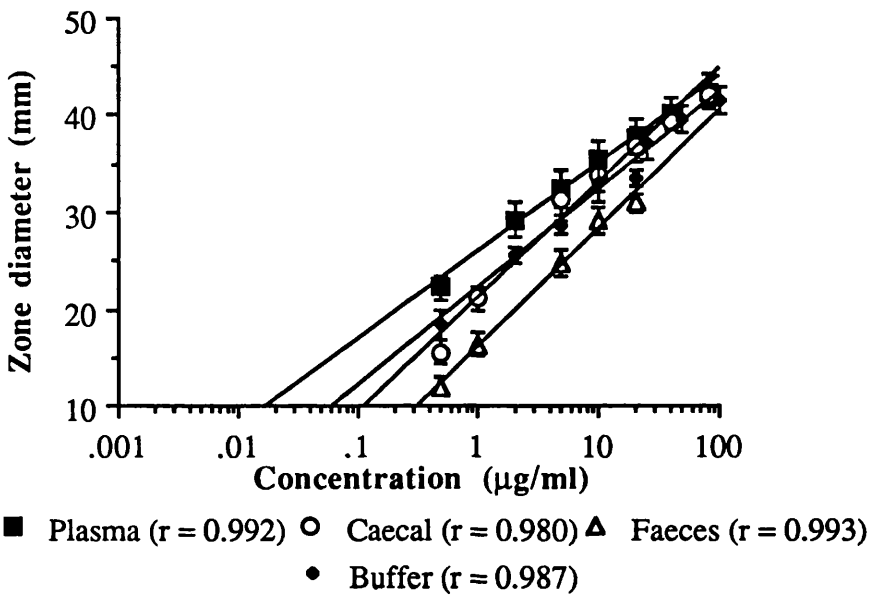

Figure 2-4. Zone diameters (mean±SD) of ampicillin sodium in equine plasma, caecal liquor, faeces and phosphate buffer

overall limit of detection, 0.07 μg/ml, was calculated from the equation of the line of best-fit, $y = 21.95 + 10.11(\log_{10}x)$.

2.10 Agar gel diffusion assay of amikacin sulphate

The agar used was the same as for penicillin G and ampicillin except that the pH of the agar was increased, from 6.6 to 7.9, by adding 5 ml of 1 M sodium hydroxide per l prior to sterilization. The test organism used was *Bacillus subtilis* (ATCC 6633). Amikacin (amikacin sulphate, Fort Dodge Laboratories Inc., Iowa, U. S. A., Willows Francis Limited, Crawley, U. K.) for standard solutions was dissolved in sterile phosphate buffer (0.05 M, pH 8.0). Plasma, caecal liquor or faecal standards were made on the day of the experiment.

Precision

The precision of the agar gel diffusion assay of amikacin sulphate in equine plasma was good as shown by the low (<9%) values of the within-day and between-day coefficients of variation of the zone diameter (mean±SD) of 4 plates analysed in a single assay and 8 plates analysed on different days (Table 2-10).

The precision of the agar gel diffusion assay of amikacin sulphate in equine caecal liquor was good as shown by the low (<10%) values of the within-day and between-day coefficients of variation of the zone diameter (mean±SD) of 4 plates analysed in a single assay and 6 or 8 plates analysed on different days (Table 2-11).

The precision of the agar gel diffusion assay of amikacin sulphate in equine faeces was good as shown by the low (<10%) values of the between-day coefficients of variation of the zone diameter (mean±SD) of 3 or 4 plates analysed on different days (Table 2-12).

Linearity and Sensitivity

The linearity of the zone diameters of amikacin sulphate was evaluated from the 12 replicate plates of standard equine plasma samples and this was found to be reasonably good ($r = 0.982$) over the range of concentrations studied (0.25-80 μg/ml) (Figure 2-5). The overall limit of detection, 0.02 μg/ml, was calculated from the equation of the line of best-fit, $y = 22.73 + 7.09(\log_{10}x)$.

The linearity of the zone diameters of amikacin sulphate was evaluated from the 12 replicate plates of standard equine caecal liquor samples and this was found to be reasonably good ($r = 0.970$) over the concentration range studied (0.25-80 μg/ml)) (Figure 2-5). The overall

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=12)	0.00 (n=4)	0.00 (n=8)
0.25	17.16±1.23 (n=12)	3.33 (n=4)	8.58 (n=8)
1	23.71±1.06 (n=12)	3.72 (n=4)	4.71 (n=8)
5	28.44±1.67 (n=12)	3.53 (n=4)	6.17 (n=8)
10	30.46±1.83 (n=12)	4.58 (n=4)	5.86 (n=8)
20	31.92±1.80 (n=12)	3.56 (n=4)	5.63 (n=8)
40	33.91±2.17 (n=8)	4.49 (n=3)	6.20 (n=5)
80	35.38±2.46 (n=8)	2.76 (n=3)	6.72 (n=5)

Table 2-10. Zone diameters and coefficients of variation of amikacin sulphate in equine plasma

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=12)	0.00 (n=4)	0.00 (n=8)
0.25	16.08±1.64 (n=12)	4.33 (n=4)	9.56 (n=8)
1	22.68±1.29 (n=12)	3.78 (n=4)	6.25 (n=8)
5	29.52±1.85 (n=12)	1.74 (n=4)	6.47 (n=8)
10	31.00±1.63 (n=12)	3.82 (n=4)	5.37 (n=8)
20	33.03±1.59 (n=12)	2.25 (n=4)	5.81 (n=8)
40	34.76±2.01 (n=10)	1.56 (n=4)	6.83 (n=6)
80	35.61±1.74 (n=10)	1.12 (n=4)	5.78 (n=6)

Table 2-11. Zone diameters and coefficients of variation of amikacin sulphate in equine caecal liquor

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of variation Between-day
0	0.00±0.00 (n=4)	0.00 (n=4)
1	15.90±1.11 (n=3)	6.98 (n=3)
5	18.87±1.47 (n=4)	7.78 (n=4)
10	21.99±2.10 (n=4)	9.56 (n=4)
20	23.64±1.57 (n=4)	6.63 (n=4)

Table 2-12. Zone diameters and coefficients of variation of amikacin sulphate in equine faeces

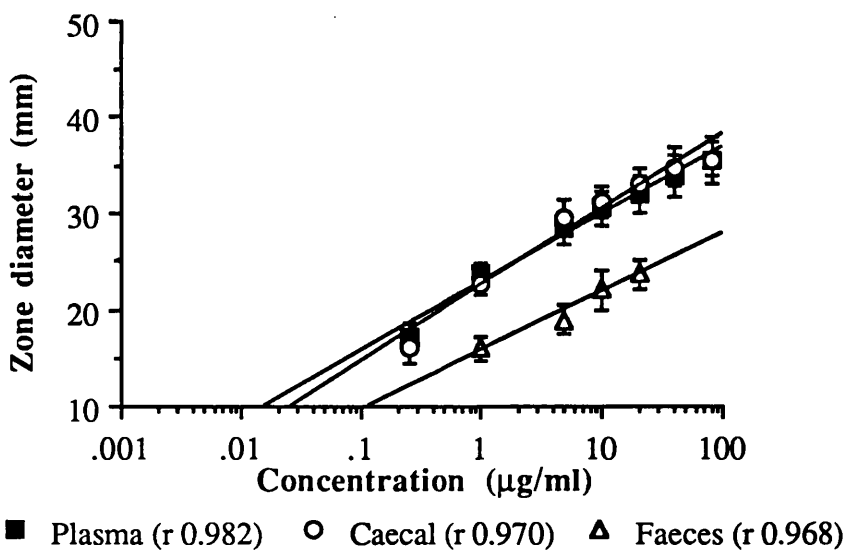

Figure 2-5. Zone diameters (mean±SD) of amikacin sulphate in equine plasma, caecal liquor and faeces

limit of detection, 0.03 μg/ml, was calculated from the equation of the line of best-fit, y = 22.31 + 7.88($\log_{10}$x).

The linearity of the zone diameters of amikacin sulphate was evaluated from the 4 replicate plates of standard equine faecal samples and this was found to be reasonably good (r = 0.968) over the concentration range studied (0.25-20 μg/ml)) (Figure 2-5). The overall limit of detection, 0.12 μg/ml, was calculated from the equation of the line of best-fit, y = 15.56 + 6.05($\log_{10}$x).

2.11 Agar gel diffusion assay of oxytetracycline hydrochloride

The test organism was *Bacillus cereus* (ATCC 11778). The test agar was 4% w/v Diagnostic Sensitivity Test agar (CM261, Oxoid, Unipath Limited, Basingstoke, U. K.) dissolved in water. Plasma, caecal liquor or faecal standards were made on the day of the experiment. Oxytetracycline (oxytetracycline hydrochloride, Sigma Chemical Company Limited, Poole, U. K.) for making standard solutions was dissolved in stock sterile phosphate buffer solution (0.05 M, pH 4.5).

Precision

The precision of the agar gel diffusion assay of oxytetracycline hydrochloride in equine plasma was good as shown by the low (<7%) within-day and between-day coefficients of variation of the zone diameter (mean±SD) of the 3 plates analysed in a single assay and 2 plates analysed on different days (Table 2-13).

The precision of the agar gel diffusion assay of oxytetracycline hydrochloride in equine caecal liquor was good as shown by the low (<9%) within-day and between-day coefficients of variation of the zone diameter (mean±SD) of the 4 plates analysed in a single assay and 8 plates analysed on different days (Table 2-14).

The precision of the agar gel diffusion assay of oxytetracycline hydrochloride in equine faeces was good as shown by the low (<6%) within-day and between-day coefficients of variation of the zone diameter (mean±SD) of the 4 plates analysed in a single assay and 5 plates analysed on different days (Table 2-15).

Linearity and Sensitivity

The linearity of the zone diameters of oxytetracycline hydrochloride was evaluated on the 5 replicate plates of standard equine plasma samples and this was found to be good (r = 0.987) over the concentration range studied (1-80 μg/ml) (Figure 2-6). The overall limit of

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00	0.00 (n=3)	0.00 (n=2)
1	17.17±0.89 (n=5)	5.00 (n=3)	5.72 (n=2)
5	25.83±1.76 (n=5)	6.99 (n=3)	6.96 (n=2)
10	28.71±1.01 (n=5)	2.98 (n=3)	4.17 (n=2)
20	31.27±1.14 (n=5)	4.71 (n=3)	1.38 (n=2)
40	33.77±1.01 (n=5)	3.10 (n=3)	3.09 (n=2)
80	36.06±1.61 (n=5)	4.48 (n=3)	4.79 (n=2)

Table 2-13. Zone diameters and coefficients of variation of oxytetracycline hydrochloride in equine plasma

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=12)	0.00 (n=4)	0.00 (n=8)
1	18.56±1.50 (n=12)	4.72 (n=4)	8.79 (n=8)
5	26.24±1.19 (n=12)	1.94 (n=4)	5.34 (n=8)
10	29.30±1.31 (n=12)	4.03 (n=4)	4.74 (n=8)
20	31.65±0.95 (n=12)	3.00 (n=4)	3.03 (n=8)
40	33.71±1.18 (n=9)	2.69 (n=3)	3.40 (n=6)
80	36.11±1.12 (n=9)	2.64 (n=3)	3.07 (n=6)

Table 2-14. Zone diameters and coefficients of variation of oxytetracycline hydrochloride in equine caecal liquor

Concentration (μg/ml)	Zone diameter (mm) (mean±SD)	Coefficient of variation (%)	
		Within-day	Between-day
0	0.00±0.00 (n=9)	0.00 (n=4)	0.00 (n=5)
1	10.33±0.50 (n=6)	3.84 (n=4)	5.79 (n=2)
4	17.07±0.72 (n=4)	4.23 (n=4)	NS
5	18.63±1.02 (n=5)	NS	1.02 (n=5)
8	20.90±0.75 (n=4)	3.60 (n=4)	NS
10	23.12±1.17 (n=5)	NS	5.06 (n=5)
20	24.82±1.13 (n=9)	3.05 (n=4)	4.00 (n=5)

Table 2-15. Zone diameters and coefficients of variation of oxytetracycline hydrochloride in equine faeces

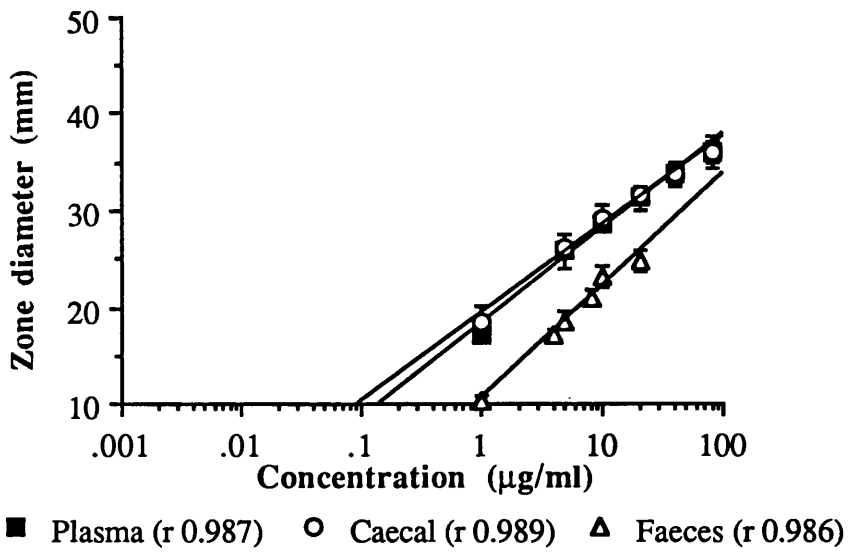

Figure 2-6. Zone diameters (mean±SD) of oxytetracycline hydrochloride in equine plasma, caecal liquor and faeces

detection, 0.15 μg/ml, was calculated from the equation of the line of best-fit, y = 18.12 + 9.85($\log_{10}$x).

The linearity of the zone diameters of oxytetracycline hydrochloride was evaluated on the 12 replicate plates of standard equine caecal liquor samples and this was found to be good (r = 0.989) over the concentration range studied (1-80 μg/ml) (Figure 2-6). The overall limit of detection, 0.10 μg/ml, was calculated from the equation of the line of best-fit, y = 19.33 + 9.15($\log_{10}$x).

The linearity of the zone diameters of oxytetracycline hydrochloride was evaluated on the 9 replicate plates of standard equine faecal samples and this was found to be good (r = 0.986) over the concentration range studied (1-20 μg/ml) (Figure 2-6). The overall limit of detection, 0.92 μg/ml, was calculated from the equation of the line of best-fit, y = 10.43 + 11.61($\log_{10}$x).

2.12 High performance liquid chromatography - General

HPLC System

A solvent delivery pump and manometric module (Gilson Model 302 and 802, Scotlab, Coatbridge, U. K.) were used to pump the solvent at an appropriate flow rate through a suitable column and a variable λ uv detector (Model CE2112, Cecil Instruments Limited, Cambridge, U. K.) set at the required λ and absorbance. The samples or standards were injected using a glass syringe into an injection port with a valve attached. The valve had a loop of similar size to the injection volume. The uv detector was connected to a variable chart recorder (Vitatron, M. S. E. Scientific Instruments, Crawley, U. K.) set at a speed of 5 mm/min and a voltage of 10 mV.

The solvent was degassed thoroughly using a sonic bath (Sonicleaner, Dawe Instruments Limited, Scotlab, Coatbridge, U. K.) and vacuum pump (Millipore U. K., Watford, U. K.) before use.

Chromatograms

The capacity factor was used to measure the time that the sample component spent in the mobile phase. The capacity factor was calculated by adjusting the retention time of the sample (sample retention time minus solvent retention time) and dividing it by the unretained peak (solvent) time. The retention time (min) of both the sample component and the unretained peak were measured (mm) from the chromatogram.

Recovery and precision

The precision of the extraction and the chromatographic procedures was assessed by the coefficients of variation of the replicate plasma samples to which the desired compounds had been added and analysed in a single assay (within-day or intra-assay variation) and on different days (between-day or inter-assay variation).

Linearity and Sensitivity

A computer programme (Cricket graph version 1.3, Cricket software, Pennsylvania, U. S. A.) was used to plot a graph of peak height (units) versus concentration (μg/ml) and was used to calculate the line of best fit and r of the replicates used for the method validation. The overall limit of detection was calculated as the concentration corresponding to a peak height of 1 unit, using the average standard peak height from the replicate analyses used in the method validation.

2.13 Estimation of oxytetracycline hydrochloride in plasma

Sample preparation

Plasma (3 ml) and 2 ml of a 10% w/v aqueous solution of trichloroacetic acid (Analar®, The British Drug House Chemicals Limited, Poole, U. K.) were added to a clean 10 ml neutral tube with a push in stopper (Sarstedt Limited, Loughborough, U. K.). The mixture was mixed well using a Griffin vortex shaker/stirrer (Griffin and George Limited, Loughborough, U. K.) and centrifuged for 15 min at 1800 *g* in a cooled centrifuge (5-15 °C) (MSE Chilspin, M. S. E. Scientific Instruments, Crawley, U. K.). The supernatant was collected for injection into a reverse phase HPLC system.

Standard solutions

Unknown concentrations of oxytetracycline hydrochloride in plasma were calculated from a standard curve of known concentrations. A standard solution of 200 μg/ml was prepared by dissolving 0.02 g of oxytetracycline (oxytetracycline hydrochloride, Sigma Chemical Company Limited, Poole, U. K.) in 100 ml of oxytetracycline eluting solution (6.12 ml orthophosphoric acid (Analar®, The British Drug House Chemicals Limited, Poole, U. K.), 2.08 g potassium dihydrogen orthophosphate (Analar®, The British Drug House Chemicals Limited) and 1.36 g 1-heptane sulphonic acid (sodium salt, Sigma Chemical Company Limited, Poole, U. K.) dissolved in 1 l of water) in a clean 100 ml glass volumetric flask. A 20 μg/ml solution was prepared by diluting 10 ml of the 200 μg/ml solution to 100 ml with oxytetracycline eluting solution. A 2 μg/ml standard solution was made by diluting 10 ml of

the 20 µg/ml solution to 100 ml in 50:50 oxytetracycline eluting solution: 10% w/v aqueous solution of trichloroacetic acid (Analar®, The British Drug House Chemicals Limited). A series of standard solutions were made, to encompass the range of likely plasma concentrations, from the standard solutions and drug-free equine plasma and put through the extraction procedure for calibration of the method and the determination of recoveries (Table 2-16).

Solvent

The solvent used contained 0.564 g 1-heptane sulfonic acid (sodium salt, Sigma Chemical Company Limited, Poole, U. K.), 5.12 ml orthophosphoric acid (Analar®, The British Drug House Chemicals Limited, Poole, U. K.), 1.72 g potassium dihydrogen phosphate (Analar®, The British Drug House Chemicals Limited) and 257 ml of acetonitrile (HPLC Grade, Rathburn Chemicals Limited, Walkerburn, U. K.) made up to 1 l with water.

Samples requiring dilution were diluted in 50:50 oxytetracycline eluting solution:10% w/v aqueous solution of trichloroacetic acid (Analar®, The British Drug House Chemicals Limited, Poole, U. K.) following extraction.

HPLC Conditions

The flow rate was set at 1 ml/min, the loop size was 20 µl and the injection volume was 15 µl. The column used was a Partisil 5 µm octadecylsilica column (125 mm long, 4.6 mm id, HPLC Technology, Macclesfield, U. K.). The uv λ was set at 354 nm and the absorbance was set at 0.02 aufs.

Chromatograms

The chromatograms of oxytetracycline hydrochloride were clearly deliniated from the solvent with a retention time of 2 min and a capacity factor of 0.57. Typical chromatograms are presented in Figure 2-7 and as can be seen, no coextracted endogenous compounds from equine plasma interfered with the oxytetracycline hydrochloride peak.

Recovery and precision

The recovery of oxytetracycline hydrochloride from equine plasma was evaluated by reference to the peak heights resulting from the direct injection of the standard solution. Twelve sets of oxytetracycline hydrochloride standards in equine plasma were extracted and chromatographed; 6 in one day and 6 on 6 different days. The recovery of oxytetracycline hydrochloride from the 12 equine plasma replicates was good and was 73.26% on average. The precision of the extraction procedure was good, as can be seen from the low values of

Concentration (µg/ml)	Standard (volume and concentration)	Plasma (ml)
0	0	3
0.25	37.5 µl of 20 µg/ml	3
1	150 µl of 20 µg/ml	3
5	75 µl of 200 µg/ml	3
10	150 µl of 200 µg/ml	3
20	300 µl of 200 µg/ml	3

Table 2-16. Standard plasma concentrations for HPLC analysis of oxytetracycline in equine plasma

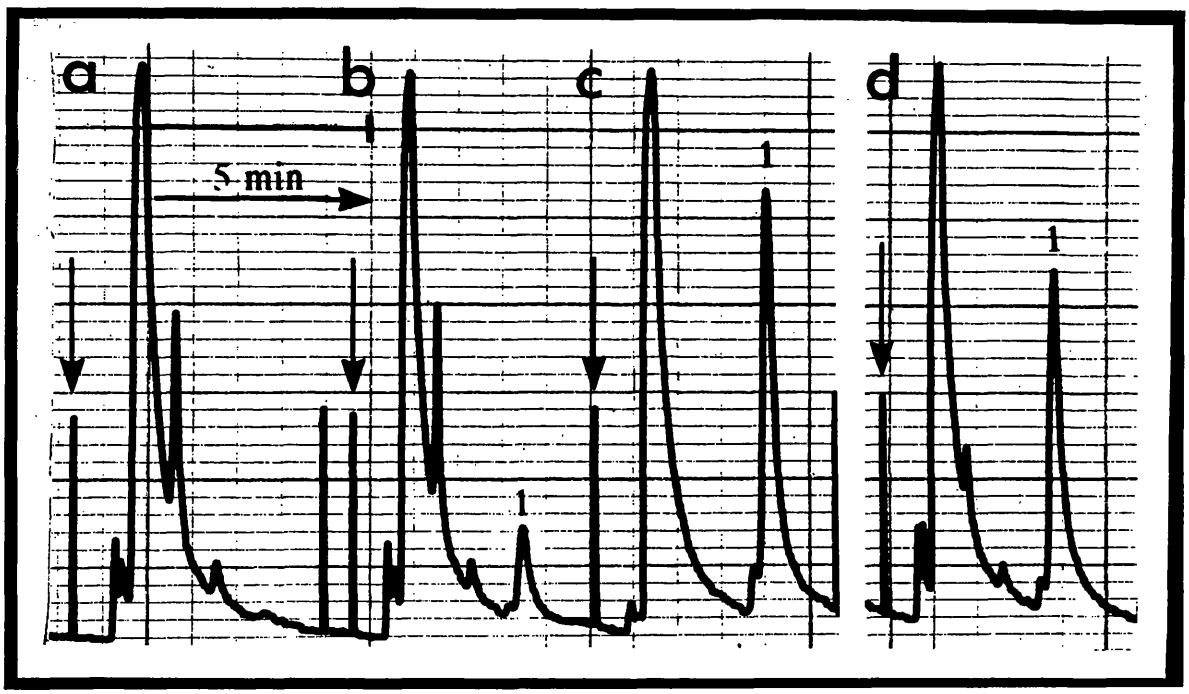

Figure 2-7. Typical chromatograms of oxytetracycline hydrochloride in equine plasma

Key: **a** drug-free plasma; **b** 1 μg/ml oxytetracycline hydrochloride in equine plasma; **c** 2 μg/ml standard solution; **d** unknown equine plasma sample (4 h after intravenous administration of oxytetracycline hydrochloride at a dose rate of 10 mg/kg bwt); ⬇ injection of sample; ➡ direction of flow; **1** oxytetracycline.

within-day and between-day coefficients of variation which averaged 8.84 and 8.14%, respectively (Table 2-17). No oxytetracycline hydrochloride was recovered from drug-free plasma.

Linearity and Sensitivity

The linearity of the plot of peak height (units) versus concentration of oxytetracycline hydrochloride (µg/ml) was evaluated on the 12 replicates of the standard equine plasma samples and this was found to be good (r = 1.000) over the concentration range studied (0.25-20 µg/ml) (Figure 2-8). The overall limit of detection of oxytetracycline hydrochloride in equine plasma, for the 12 replicate analyses, was 0.08 µg/ml.

2.14 Estimation of SCFA in equine caecal liquor and faeces

An HPLC method, using an ion-exchange column similar to the one used by Guerrant *et al.* (1982), was used to measure SCFA in equine caecal liquor and faeces.

Sample preparation

Caecal liquor (1 ml) was passed through a Sep-pak C18 cartridge (Waters, Millipore U. K., Dagenham, U. K.) which had been pre-conditioned with 5 ml methanol (HPLC Grade, Rathburn Chemicals Limited, Walkerburn, U. K.) and 5 ml 0.02 M chloride buffer (pH 1.9). Excess fluid was shaken out of the Sep-pak cartridge prior to elution. The eluent was 3.5 ml of phosphate buffer (0.05 M, pH 7.0). After elution, 3.5 ml was collected and filtered through a 0.22 µm prefilter (AP25, Millipore U. K.) and a 0.45 µm white cellulose nitrate membrane filter (25 mm diameter, Whatman Labsales Limited, Maidstone, U. K.) in a reusable filter holder (25 mm diameter, Swinnex, Millipore U. K.). The filter was pre-conditioned with 3 ml phosphate buffer (0.05 M, pH 7.0) and washed with 2 ml phosphate buffer (0.05 M, pH 7.0). 20 µl of the resultant solution (total volume 5.5 ml) was injected into an ion exchange chromatograph.

A 1-2 g aliquot of each faecal was weighed into a 10 ml neutral tube with a push in stopper (Sarstedt Limited, Loughborough, U. K.) and 3 times the mass of phosphate buffer (0.05 M, pH 7.0) was added. The sample/phosphate buffer was mixed thoroughly for 20-30 s using a Griffin vortex shaker/stirrer (Griffin and George Limited, Loughborough, U. K.), centrifuged for 15 min at 1800 *g* in a cooled centrifuge (5-15 °C) (MSE Chilspin, M. S. E. Scientific Instruments, Crawley, U. K.), and the resultant supernatant passed through the extraction procedure.

Concentration (μg/ml)	Recovery (%)	Coefficient of Variation (%)	
		Within-day	Between-day
0.25	77.33 (n=11)	5.97 (n=5)	8.65 (n=6)
1	65.08 (n=12)	9.15 (n=6)	7.44 (n=6)
5	73.97 (n=12)	1.91 (n=6)	2.55 (n=6)
10	78.03 (n=12)	1.75 (n=6)	7.93 (n=6)
20	72.21 (n=12)	2.54 (n=6)	5.52 (n=6)
Mean	73.26 (n=59)	8.84 (n=29)	8.14 (n=30)

Table 2-17. Mean recovery and coefficients of variation of oxytetracycline hydrochloride in equine plasma

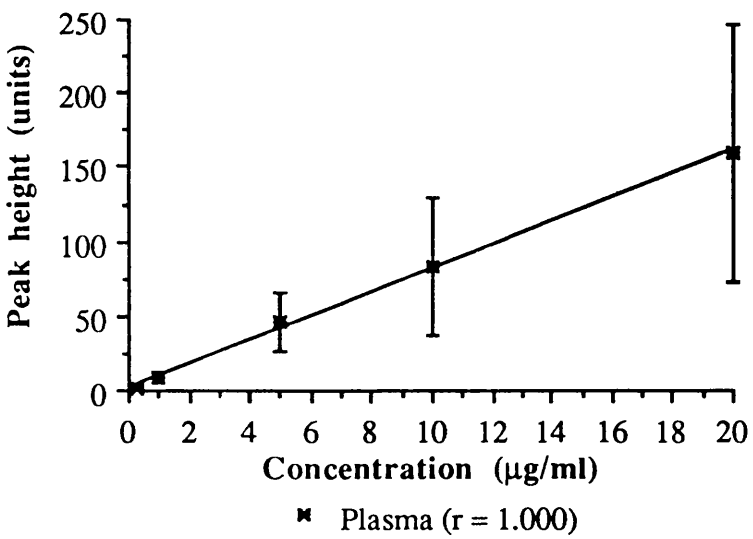

Figure 2-8. Peak heights (mean±SD) of oxytetracycline hydrochloride in equine plasma

Standard solutions

Stock solutions of 0.5 M lactic (Analar®, not less than 88%, The British Drug House Chemicals Limited, Poole, U. K.), acetic (HPLC Grade, The British Drug House Chemicals Limited), propionic (approximately 98%, Sigma Chemical Company Limited, Poole, U. K.), isobutyric (2-methyl propionic acid, approximately 98%, Sigma Chemical Company Limited) and butyric (approximately 98%, Sigma Chemical Company Limited) acids were prepared by diluting 0.75, 0.57, 0.75, 0.93 and 0.92 ml, respectively, in 20 ml water. Similarly 0.1 M stock solutions of isovaleric (3-methyl butyric acid, approximately 98%, Sigma Chemical Company Limited) and valeric (approximately 98%, Sigma Chemical Company Limited) acids were prepared by diluting 0.22 ml of each in 20 ml of water. A composite standard, containing all 7 SCFA, was prepared from the stock solutions diluted in phosphate buffer (0.05 M, pH 7.0) to a concentration 0.001 M. A series of standard samples (Table 2-18) was made from the standard solutions and phosphate buffer (0.05 M, pH 7.0), to encompass the range of likely caecal liquor or faecal concentrations, and put through the extraction procedure for calibration and determination of recovery.

Solvent

The mobile phase was 0.05 M sulphuric acid (1.35 ml diluted to 1 l with water, apparent pH 2.0, Pronalys*AR, analytical grade, May and Baker Limited, Dagenham, U. K.).

HPLC Conditions

The pump flow rate was set at 0.5 ml/min and the loop size and injection volume were 50 µl. A Brownlee Polypor H (10 µm) column (250 mm long, 4.6 mm id, Anachem Limited, Luton, U. K.) for the separation of weak organic acids was used. The uv λ was set at 210 nm and the absorbance at 0.02 aufs.

Chromatograms

All seven SCFA formed well delineated, distinct peaks with retention times of 4.8 (L), 6.0 (A), 6.9 (P), 7.6 (IB), 8.6 (B), 9.9 (IV), and 12.8 (V) min and capacity factors of 0.85 (L), 1.31 (A), 1.65 (P), 1.92 (IB), 2.31 (B), 2.81 (IV), and 3.92 (V). Typical chromatograms are presented in Figure 2-9 and, as can be seen, no coextracted endogenous compounds from caecal liquor interfered with the acid peaks.

Recovery and precision

The recovery of the 7 SCFAs from phosphate buffer, to which the 7 SCFAs had been added, was calculated by reference to the peak heights resulting from direct injection of the

Concentration (mmol/l)	Standard (volume and concentration)	Phosphate buffer (ml)
0.0	0	10.00
0.5	10 µl of 0.5 M; 50 µl of 0.1 M	9.85
1.0	20 µl of 0.5 M; 100 µl of 0.1 M	9.70
2.5	50 µl of 0.5 M; 250 µl of 0.1 M	9.25
5.0	100 µl of 0.5 M; 500 µl of 0.1 M	4.25
10.0	200 µl of 0.5 M; 1 ml of 0.1 M	3.50
50.0	1 ml of 0.5 M; no 0.1 M	2.50

Table 2-18. Standard phosphate buffer concentrations for HPLC analyses of SCFA

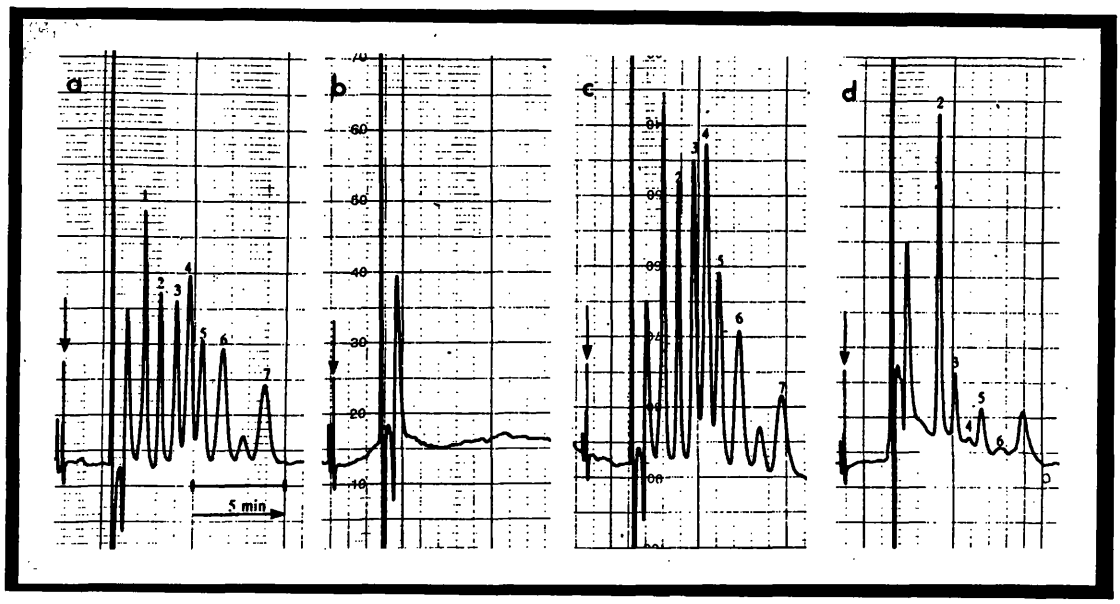

Figure 2-9. Typical chromatograms of 7 SCFA in phosphate buffer and equine caecal liquor

Key: **a** 1.0 mmol/l standard; **b** SCFA-free phosphate buffer; **c** 10.0 mmol/l SCFA in phosphate buffer; **d** SCFA in equine caecal liquor 6 h after oral administration of ampicillin sodium at a dose rate of 10 mg/kg bwt; ⬇ injection of sample; ➡ direction of flow; **1** L; **2** A; **3** P; **4** IB; **5** B; **6** IV; **7** V.

composite standard solution. Recovery of the 7 SCFA from phosphate buffer was good and was 74.30 (L), 76.76 (A), 84.48 (P), 84.42 (IB), 82.55 (B), 79.43 (IV), and 72.56 (V) % on average (Tables 2-19a and 2-19b). No SCFA were recovered from SCFA-free phosphate buffer.

The precision of the extraction method was reasonable, as shown by the good to moderate values of the within-day (6 replicates) coefficients of variation which averaged 11.07 (L), 10.41 (A), 7.63 (P), 16.58 (IB), 18.67 (B), 15.06 (IV) and 20.90 (V) and the between-day (6 replicates) coefficients of variation which averaged 12.02 (L), 12.14 (A), 10.75 (P), 16.25 (IB), 14.79 (B), 15.56 (IV) and 17.80 (V) (Tables 2-19a and 2-19b).

Linearity and Sensitivity

The linearity of peak height versus concentration of the 7 SCFA was evaluated from the 12 replicates of the standard phosphate buffer samples and this was found to be good ($r = 0.996$ (L), $r = 0.992$ (A), $r = 0.996$ (P), $r = 1.000$ (IB), $r = 1.000$ (B), $r = 0.990$ (IV) and $r = 0.982$ (V)) over the concentration range studied (0.5-10.0 mmol/l) (Figure 2-10). The 50 mmol/l phosphate buffer standard was validated at a later date and was not included here. The overall limit of detection of the seven acids in phosphate buffer, *i. e.* the concentration corresponding to a peak height of 1 unit, was calculated using the average standard peak height for the 12 replicate analyses and was 0.1 mmol/l (L, A, P and IB) and 0.2 mmol/l (B, IV and V).

2.15 Bacteriological examinations

Smear preparation and staining technique

An air dried heat fixed smear was made from each sample and stained by Gram's method. The smear was covered with 0.5% w/v aqueous solution of crystal violet (The British Drug House Chemicals Limited, Poole, U. K.) for 30 s then rinsed off with tap water, this was replaced by Gram's iodine (1 g iodine resublimed GPR (The British Drug House Chemicals Limited) and 2 g potassium iodide (The British Drug House Chemicals Limited) dissolved in 300 ml water) for 30 s then rinsed off with tap water, decolourized with acetone (technical grade, The British Drug House Chemicals Limited) for 1-2 s, washed with tap water, counterstained for 30 s with dilute carbol fuschin (1:10 with water, fuschin basic, The British Drug House Chemicals Limited), washed with water and blotted dry. Examination of stained smears was by oil immersion (Lenzol immersion oil, The British Drug House Chemicals Limited) light microscopy at 1000 times magnification (SM Lux, Ernst Leitz GmBH, Wetzlar, Germany) and organisms were identified by their Gram stain reaction (positive purple; negative pink) and morphology (coccus or bacillus).

Acid	Concentration (mmol/l)	Recovery (%)	Coefficient of Variation (%) Within-day	Between-day
L	0.5	82.97 (n=12)	7.11 (n=6)	12.88 (n=6)
	1.0	71.30 (n=12)	10.87 (n=6)	8.21 (n=6)
	2.5	72.65 (n=12)	7.42 (n=6)	11.36 (n=6)
	5.0	69.82 (n=12)	8.87 (n=6)	15.70 (n=6)
	10.0	77.69 (n=12)	5.48 (n=6)	8.22 (n=6)
	50.0	71.14 (n=11)	7.63 (n=5)	7.17 (n=6)
	Mean	**74.30 (n=71)**	**11.07 (n=35)**	**12.02 (n=36)**
A	0.5	76.25 (n=12)	9.45 (n=6)	17.68 (n=6)
	1.0	74.44 (n=12)	8.81 (n=6)	7.94 (n=6)
	2.5	73.86 (n=12)	9.13 (n=6)	8.87 (n=6)
	5.0	74.90 (n=12)	7.63 (n=6)	13.17 (n=6)
	10.0	86.56 (n=12)	5.55 (n=6)	2.51 (n=6)
	50.0	74.38 (n=11)	6.96 (n=5)	11.86 (n=6)
	Mean	**76.76 (n=71)**	**10.41 (n=35)**	**12.14 (n=36)**
P	0.5	80.23 (n=11)	9.67 (n=6)	15.16 (n=5)
	1.0	83.37 (n=12)	6.74 (n=6)	12.21 (n=6)
	2.5	79.92 (n=12)	5.05 (n=6)	8.42 (n=6)
	5.0	85.79 (n=12)	5.93 (n=6)	10.30 (n=6)
	10.0	93.06 (n=11)	4.10 (n=5)	2.07 (n=6)
	50.0	84.93 (n=11)	7.20 (n=5)	7.02 (n=6)
	Mean	**84.48 (n=69)**	**7.63 (n=34)**	**10.75 (n=35)**

Table 2-19a. Mean recovery and coefficients of variation of 7 SCFA in phosphate buffer

Acid	Concentration (mmol/l)	Recovery (%)	Coefficient of Variation (%) Within-day	Between-day
IB	0.5	87.01 (n=12)	7.83 (n=6)	12.22 (n=6)
	1.0	87.60 (n=12)	12.38 (n=6)	11.99 (n=6)
	2.5	91.59 (n=12)	6.88 (n=6)	10.49 (n=6)
	5.0	91.98 (n=12)	6.61 (n=6)	10.14 (n=6)
	10.0	88.36 (n=12)	6.69 (n=6)	4.45 (n=6)
	50.0	57.75 (n=11)	11.22 (n=5)	5.02 (n=6)
	Mean	**84.42 (n=71)**	**16.58 (n=35)**	**16.25 (n=36)**
B	0.5	82.31 (n=11)	10.10 (n=6)	17.08 (n=6)
	1.0	87.35 (n=12)	10.20 (n=6)	8.29 (n=6)
	2.5	90.09 (n=12)	5.78 (n=6)	9.68 (n=6)
	5.0	88.30 (n=11)	9.93 (n=5)	13.75 (n=6)
	10.0	87.82 (n=12)	5.21 (n=6)	3.11 (n=6)
	50.0	57.82 (n=11)	14.07 (n=5)	8.51 (n=6)
	Mean	**82.55 (n=69)**	**18.67 (n=34)**	**14.79 (n=35)**
IV	0.5	86.52 (n=10)	15.14 (n=5)	15.77 (n=5)
	1.0	86.02 (n=11)	5.01 (n=5)	12.33 (n=6)
	2.5	87.54 (n=12)	5.43 (n=6)	9.41 (n=6)
	5.0	74.49 (n=12)	8.99 (n=6)	12.46 (n=6)
	10.0	64.82 (n=12)	6.12 (n=6)	4.63 (n=6)
	Mean	**79.43 (n=57)**	**15.06 (n=28)**	**15.56 (n=29)**
V	0.5	84.91 (n=11)	4.28 (n=5)	16.72 (n=6)
	1.0	72.19 (n=11)	17.41 (n=5)	16.52 (n=6)
	2.5	83.89 (n=12)	14.47 (n=6)	9.40 (n=6)
	5.0	67.14 (n=12)	6.56 (n=6)	10.16 (n=6)
	10.0	56.08 (n=12)	13.57 (n=6)	1.88 (n=6)
	Mean	**72.56 (n=58)**	**20.90 (n=28)**	**17.80 (n=30)**

Table 2-19b. Mean recovery and coefficients of variation of 7 SCFA in phosphate buffer

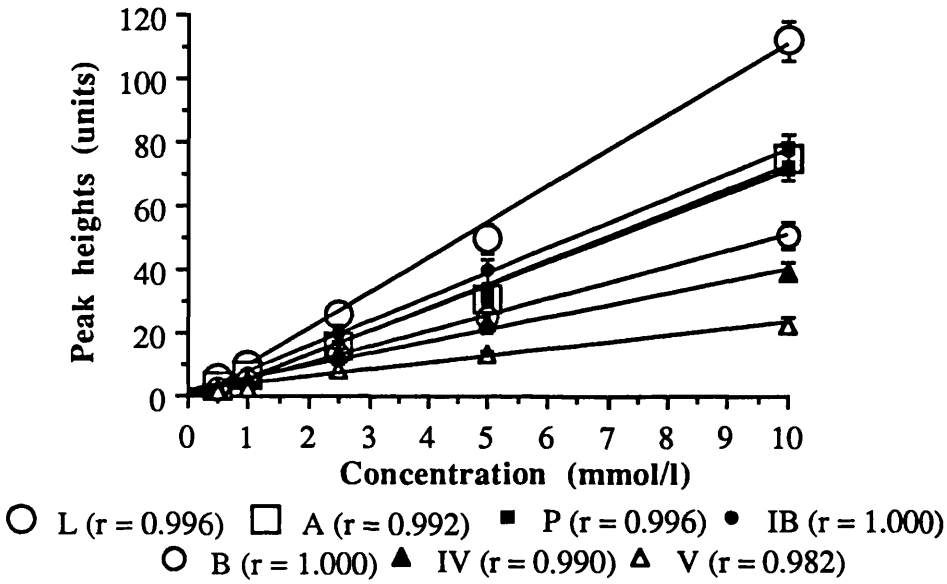

Figure 2-10. Peak heights (mean±SD) of 7 SCFA in phosphate buffer

Technique for counting of viable cfu

Eight ten-fold dilutions of 1.0 ml of caecal liquor or 1.0 g of faecal material were prepared in sterile phosphate buffered saline (pH 7.3, Dulbecco 'A', BR14a, Oxoid, Unipath, Basingstoke, U. K.). A 10 µl drop of each dilution was placed onto agar plates, which had been marked out as a template of 8 segments, and allowed to dry. The limit of detection was 10^3 viable cfu/ml or /g, *i. e.* 1 viable colony in the first dilution. In Appendices A, B, C and D the counts are expressed in scientific notation, where 1.00E+03 equals $1x10^3$.

Material obtained from each sample was used to inoculate a 7% sheep blood (210101, Becton-Dickinson, Dublin, Ireland) agar plate (CM271, Oxoid, Unipath, Basingstoke, U. K.) to detect coliforms, a MacConkey agar plate (CM7, Oxoid, Unipath) to detect non-lactose fermenting aerobic bacteria and a 7% horse blood (SR50, Oxoid, Unipath) agar plate (CM271, Oxoid, Unipath) to detect *Bacteroides spp.*. Slanetz and Bartley (S&B) agar (CM377, Oxoid, Unipath) was inoculated to detect faecal streptococci and Mann, Sharpe and deRogosa (MRS) agar (CM361, Oxoid, Unipath) was inoculated to detect lactobacilli. Perfringens agar base (CM587, Oxoid, Unipath) supplemented with TSC supplement (SR88, Oxoid, Unipath) was inoculated to detect *C. perfringens*. *Clostridium difficile* agar base (CM601, Oxoid, Unipath) supplemented with selenium (SR96, Oxoid, Unipath) and 7% horse blood was inoculated to detect the presence of *C. difficile*.

The inoculated sheep blood agar, MacConkey agar and S&B agar plates were incubated aerobically (LTE Qualitemp 80, Laboratory Thermal Equipment, Oldham, U. K.) at 37 °C and the MRS agar plates were incubated microaerophilically in an atmosphere of 5% carbon dioxide and 95% hydrogen (BOC, Glasgow, U. K.) at 37 °C in a McIntosh and Fildes' anaerobic jar (Don Whitley Scientific Limited, Shipley, U. K.) with no cold catalyst added. In the initial experiments, anaerobic plates were incubated at 37 °C in an atmosphere of 5% carbon dioxide and 95% hydrogen (BOC) in a McIntosh and Fildes' anaerobic jar, with 2 cold catalysts (D catalyst, Engelhard, Cinderford, U. K.) added. The remaining plates were incubated anaerobically in an environment containing 10% hydrogen (BOC), 10% carbon dioxide (BOC) and 80% nitrogen (BOC) in a MK3 Anaerobic Workstation (Don Whitley Scientific Limited) at 37 °C. Air was removed from the McIntosh and Fildes' anaerobic jars using an oil free vacuum pump and compressor (Edwards, Crawley, U. K.) with air removal stopped at 500 mm Hg for the microaerophilic jars and 600 mm Hg for the anaerobic jars. Plates that were incubated aerobically were examined after 24 h incubation, microaerophilic plates after 48 h incubation and anaerobic plates after 72 h incubation at 37 °C. The anaerobic media were incubated for up to 72 h because some of the organisms were slow growing, and pigment producing organisms (such as some *Bacteroides spp.*) took longer than 24-48 h to produce pigment. The bacterial colonies present on each medium

were recorded, identified presumptively by their colonial morphology and Gram stain reaction, and subcultured for subsequent identification. The resultant colonies on the segmented plates were counted, recorded and the numbers of each organism calculated. An example of a typical plate is shown in Figure 2-11.

A single colony of an anaerobic bacterium for identification was subcultured onto horse blood agar and incubated anaerobically for 24-72 h at 37 °C. A rapid biochemical identification was carried out using a system for the identification of anaerobes (API20A, bioMérieux UK Limited, Basingstoke, U. K.) and used along with other tests, such as colonial and microscopic morphology and Gram stain reaction, to confirm the identification. The test system contains 20 microtubules of dehydrated test substances. Bacterial suspension is dispensed into the microtubules and the bacterial metabolites produced by anaerobic incubation result in an alteration in the colour of the reconstituted substrates, due to either a change in pH or the addition of specific reagents. The substrates are tryptophan, urea, glucose, mannitol, lactose, saccharose, maltose, salicin, xylose, arabinose, gelatin, esculin/ferric citrate, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose.

The growth from a subculture plate was harvested using a plain sterile swab (Medical Wire and Equipment Company (Bath) Limited, Corsham, U. K.) and emulsified in a freshly opened ampoule of API20A medium (bioMérieux UK Limited), taking care to avoid dissolving air in the inoculation medium, to produce a final turbidity of greater than or equal to tube 3 on the McFarland scale. An individual incubation box was moistened with 5 ml sterile water and an individual API20A strip (bioMérieux UK Limited) inserted. Each tube on the strip was filled with the inoculated API20A medium (bioMérieux UK Limited) using a sterile Pasteur micropipette. Care was taken to avoid air bubble formation in the tubes by tilting the strip during inoculation. The tube and cupule were filled with the test suspension to detect hydrolysis of gelatin or protease production. The cupule of the test for indole formation (tryptophan) was filled with mineral oil (bioMérieux UK Limited) to prevent evaporation of any indole produced. After inoculation, the incubation box was closed and the test strip incubated for 24-48 h in the MK3 anaerobic workstation at 37 °C.

After 24 h incubation, reactions which do not require the addition of reagents, namely, the production of urease (positive red; negative yellow-orange), the acidification of sugars (positive yellow/green-yellow; negative purple), and the hydrolysis of gelatin (positive diffusion of black pigment; negative no pigment diffusion) and esculin (positive brown-black; negative yellow) were read, and if reactions were observed reagents were added to the remaining tubes. If only a few or no reactions were observed the strip was incubated for a further 24 h. Indole formation was detected by adding a drop of XYL reagent (xylene,

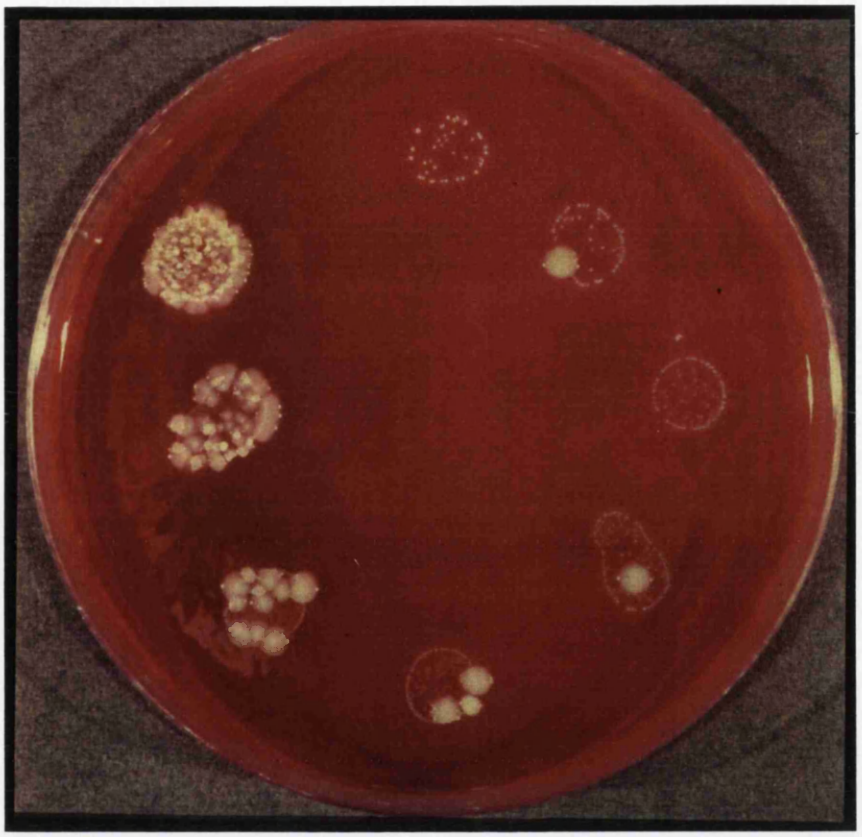

Figure 2-11. A typical example of a plate showing a count of viable coliforms using serial dilutions

bioMérieux UK Limited) to the mineral oil in the tryptophan cupule, mixing it and leaving it for 2-3 min then adding a drop of EHR reagent (8.75% w/v solution of *p*-dimethylaminobenzaldehyde in 82.5:17.5 ethanol:37% hydrochloric acid, bioMérieux UK Limited). The reagent floated on the surface of the mineral oil and a reaction (positive red; negative yellow) took place within 5 min. A drop of BCP reagent (0.02% w/v aqueous solution of bromocresol purple, bioMérieux UK Limited) was added to the sugar containing tubes if they were difficult to interpret due to discolouration caused by reduction; discoloured tests were recorded as a negative reaction. Organisms that produce hydrogen sulphide produced a black precipitate of ferric sulphide at the base of the esculin/ferric citrate tube, whereas esculin hydrolysis resulted in a brown-black area at the top of the tube. In cases where the esculin/ferric citrate tube was entirely brown-black, the strip was placed under a uv lamp at λ 365 nm (Wood's lamp) to detect hydrogen sulphide production (positive no fluorescence; negative fluorescence). Production of catalase (positive gas bubble production; negative no gas production) was tested by adding 2 drops of a 30% aqueous solution of hydrogen peroxide (100 volumes, Fisons Limited, Loughborough, U. K.) to the mannitol tube after the strip had been in an aerobic environment for 30 min. In addition, Gram stain reaction, microscopic morphology and presence or absence of spores were recorded. Positive and negative reactions were recorded on a report sheet (bioMérieux UK Limited) and compared with the results in the Analytical Profile Index (bioMérieux UK Limited). A typical example of a biochemical identification strip is shown in Figure 2-12.

Selective isolation of *Salmonella spp.* was by inoculation of 10 ml of tetrathionate broth (CM29, Oxoid, Unipath, Basingstoke, U. K.) with either 1 ml of caecal liquor or a sterile loop of faecal material, addition of 200 µl of aqueous iodine solution (30 g iodine resublimed GPR (The British Drug House Chemicals Limited) and 25 g potassium iodide (The British Drug House Chemicals Limited) dissolved in 100 ml water), mixing by gentle rotation and aerobic incubation. After 24 h incubation, both *Salmonella/Shigella* modified agar (CM533, Oxoid, Unipath) and desoxycholate citrate (Hynes modification) agar (CM227, Oxoid, Unipath) were inoculated from the tetrathionate broth and incubated aerobically for 24 h. A 1 ml aliquot of urea broth (urea broth base, CM271, supplemented with 40% urea, SR020K, Oxoid, Unipath) was inoculated with a single suspect colony (yellow/translucent) from the agar plates and any urease negative organisms (positive pink; negative yellow/translucent), after 24 h aerobic incubation, were inoculated onto a TSI agar slant (CM 277, Oxoid, Unipath) for subsequent identification. Reactions of organisms on TSI slant were interpreted using a table of the reaction of Gram negative bacteria on TSI slants (Carter, 1979) after 24 h aerobic incubation (Figure 2-13).

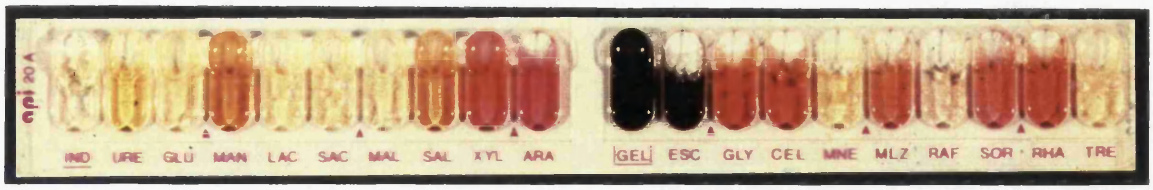

Figure 2-12. A typical example of a rapid biochemical identification strip of *C. perfringens*

Key: positive reaction (+), negative reaction (-); **IND** (+) indole, **URE** (-) urease, **GLU** (+) glucose, **MAN** (-) mannitol, **LAC** (+) lactose, **SAC** (+) saccharose, **MAL** (+) maltose, **SAL** (+) salicin, **XYL** (-) xylose, **ARA** (-) arabinose, **GEL** (+) gelatin, **ESC** (-) esculin, **GLY** (-) glycerol, **CEL** (-) cellobiose, **MNE** (+) mannose, **MLZ** (-) melezitose, **RAF** (-) raffinose, **SOR** (-) sorbitol, **RHA** (-) rhamnose, **TRE** (+) trehalose.

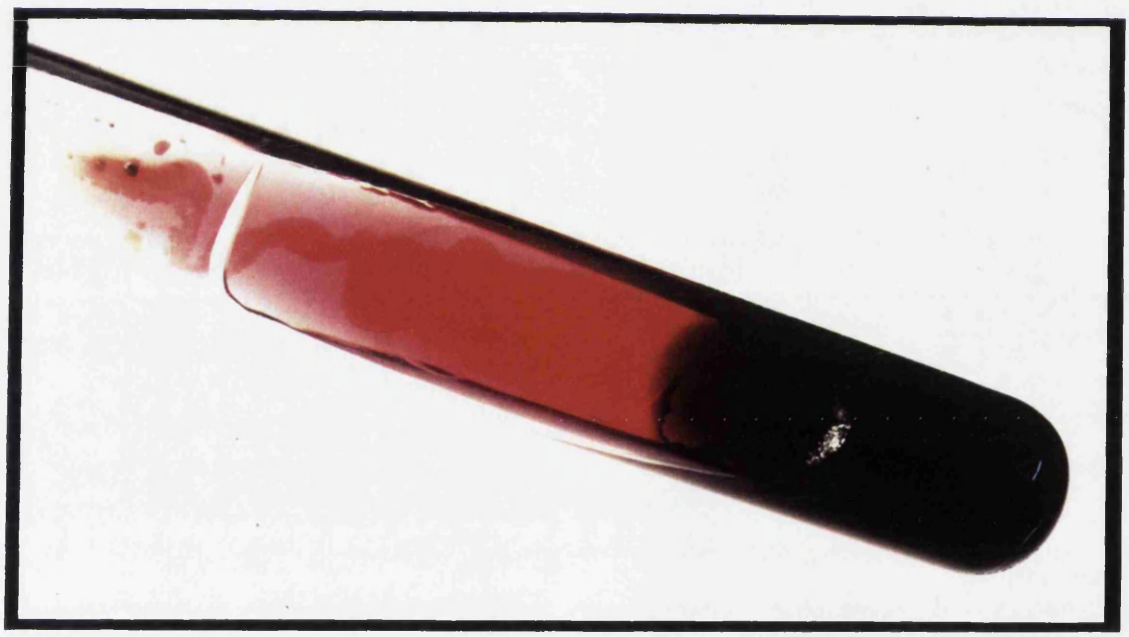

Figure 2-13. A typical TSI slant from an isolate of *Salmonella typhimurium* phage type 204c

Key: red alkaline slant; black hydrogen sulphide production

Any suspect *Salmonella spp.* were tested serologically for both somatic (O) and flagellar (H) antigens using polyvalent O (groups A-G), polyvalent H (phase 1 and 2) and subgroups B, C and D *Salmonella* agglutinating sera (Wellcome Diagnostics, Dartford, U. K.). A suspect colony was emulsified in two drops of sterile water on a clean glass slide. A drop of agglutinating serum was added to one and the drops mixed by gentle rotation of the slide. A positive agglutination (appearance of clumps of material in drop) occurred rapidly (within 2 min). The drop with no agglutinating serum added was used as a control for autoagglutination.

Where positive agglutinations occurred a standardized biochemical identification system for the identification of *Enterobacteria* and other Gram negative rods (API20E, bioMérieux UK Limited, Basingstoke, U. K.) was used for further identification. Bacterial metabolism was indicated by either a pH indicator in the substrate microtubules or by the addition of specific reagents after a suitable period of incubation.

A single colony subcultured onto a 7% sheep blood (210101, Becton-Dickinson, Dublin, Ireland) agar plate (CM271, Oxoid, Unipath, Basingstoke, U. K.) was harvested using a sterile wire loop and emulsified in a 5 ml of sterile water to produce a homogeneous suspension. An individual incubation box was moistened with 5 ml of sterile water and an individual API20E strip (bioMérieux UK Limited) inserted. Each tube on the strip was filled with the inoculated sterile water using a sterile Pasteur micropipette. Care was taken to avoid air bubble formation in the tubes by tilting the strip during inoculation. The tube and cupule were filled with the test suspension to detect citrate utilization (sodium citrate, positive blue-green/green; negative pale green/yellow), hydrolysis of gelatin (Kohn's gelatin, positive diffusion of black pigment; negative no pigment diffusion) or acetoin production (sodium pyruvate). Only the tube was filled for the tests for production of ß-galactosidase (*o*-nitrophenylgalactoside, positive yellow; negative colourless), arginine dihydrolase (arginine, positive red/orange; negative yellow), lysine decarboxylase (lysine, positive orange; negative yellow), ornithine decarboxylase (ornithine, positive red/orange; negative yellow), sodium thiosulphate (hydrogen sulphide production, positive black deposit/thin line; negative colourless/greyish), tryptophan desaminase and indole (tryptophan) and for fermentation or oxidation sugars (positive yellow; negative blue/blue green) glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In addition, the cupules of the tubes of arginine, lysine, ornithine, urea and hydrogen sulphide production were filled with mineral oil (bioMérieux UK Limited) to create an anaerobic environment. After inoculation, the incubation box was closed and the test strip incubated aerobically at 37 °C for 18-24 h. After 18-24 h incubation, if glucose (positive yellow; negative blue/blue-green) was fermented or 3 or more tests were positive, the reagents were

added to the remaining tests. The Voges Proskauer (VP) test (positive pink/red; negative colourless) for acetoin was performed by adding a drop of reagents VP1 (40% w/v aqueous solution of potassium hydroxide, bioMérieux UK Limited) and VP2 (6% α naphthol in ethanol, bioMérieux UK Limited) and waiting for at least 10 min. The tryptophan deaminase (TDA) test (positive dark brown; negative yellow) was carried out by adding a drop of TDA reagent (3.4% w/v aqueous solution of ferric chloride, bioMérieux UK Limited) to the tryptophan tube. Indole (IND) formation (positive red ring; negative yellow ring) was detected by adding a drop of IND reagent (5% *p*-dimethylaminobenzaldehyde dissolved in a 75:25 isoamyl alcohol:37% hydrochloric acid, bioMérieux UK Limited). A nitrite (NIT) test (positive red; negative yellow) was performed by adding 1 drop of NIT1 (0.8% sulfanilic acid in acetic acid, bioMérieux UK Limited) and NIT2 (0.6% N-N-dimethyl-1-naphthylamine in 5 M acetic acid, bioMérieux UK Limited) to the glucose tube and waiting for 2-3 min. A negative reaction occurred if reduction occurred in the tube. In addition, the results of an oxidase reaction and a Gram stain reaction, and the microscopic morphology were recorded. The oxidase test (positive purple; negative colourless) was carried out by wetting a piece of filter paper with 0.5% w/v aqueous solution of N-tetramethyl-*p*-phenylene-diamine dihydrochloride (The British Drug House Chemicals Limited, Poole, U. K.) and emulsifying a single colony on the strip of paper. Positive and negative reactions were recorded on a report sheet (bioMérieux UK Limited) and compared with the results in the Analytical Profile Index (bioMérieux UK Limited). *Salmonella spp.* were sent to a reference laboratory (National Health Service (Scotland) *Salmonella* Reference Laboratory, Stobhill General Hospital, Glasgow, U. K.) for antigenic analysis to identify the species and phage type.

The susceptibility of any *Salmonella spp.* isolated, to a series of standard antimicrobial agents, was examined *in vitro*. A plate of diagnostic sensitivity test agar (CM261, Oxoid, Unipath Limited, Basingstoke, U. K.) with 7% horse blood (SR50, Oxoid, Unipath) added was inoculated with the test organism. A series of 8 paper susceptibility discs (Oxoid, Unipath Limited, Basingstoke, U. K.) containing antimicrobial agents (10 μg ampicillin, 30 μg chloramphenicol, 50 μg furazolidone, 10 μg neomycin, 30 μg oxytetracycline, 10 μg streptomycin, 100 μg sulfafurazole and 1.25 μg/23.75 μg trimethoprim/ sulphamethoxazole) were spaced evenly on the surface of the agar and the plate was incubated aerobically for 24 h. Disks with a clear zone of inhibition of bacterial growth were read as positive and where there was no zone of growth inhibition the bacterium was considered to be resistant to that antimicrobial agent.

Screening for *Salmonella* species

Selective isolation of *Salmonella* species from 12 ponies at slaughter was carried out by inoculating 10 ml of tetrathionate broth with either a plain sterile swab (Medical Wire and Equipment Company (Bath) Limited, Corsham, U. K.) taken from the terminal ileal or caecal mucosa, or a portion of mesenteric lymph node. After 24 h incubation, both *Salmonella/Shigella* modified agar (CM533, Oxoid, Unipath) and desoxycholate citrate (Hynes modification) agar (CM227, Oxoid, Unipath) were inoculated from the tetrathionate broth and incubated aerobically for 24 h.

2.16 Caecal pH

The pH of serial samples of caecal liquor was measured using a hand held Whatman® pH μsensor (Whatman Labsales Limited, Maidstone, U. K.). The pH meter was calibrated to pH 7.0 using pH 7.0 buffer (±0.01 at 20°C, The British Drug House Chemicals Limited, Poole, U. K.). The pH meter was calibrated between measurements, after washing with deionized water (Ionmiser, Model 2C, Houseman Hegro, Slough, U. K.) and blotting dry.

2.17 Faecal dry matter

Faecal dry matter content was estimated as a means of monitoring the development of diarrhoea.

An aliquot of 1-2 g of faeces was weighed from each sample and put into a 5 ml neutral tube (Sarstedt Limited, Loughborough, U. K.), the mass of the tube and the tube plus sample were recorded. Each aliquot was taken from the middle of a faecal ball. Uncovered tubes were placed in a polystyrene rack and the whole was covered with aluminium foil and placed in an oven (Laboratory Thermal Equipment, Oldham, U. K.) at 50-60 °C. Drying was monitored by weighing the tubes after 48, 72 and 120 h. The initial mass of each sample was calculated by subtracting the mass of the tube (g) from the mass of the tube plus sample (g). After drying the mass of faeces remaining was calculated as a percentage of the initial mass of each aliquot.

2.18 Plasma biochemistry

Estimation of plasma sodium and potassium ion concentrations was carried out using atomic emission spectrophotometry (Flame Photometer 543, Instrumentation Laboratories, Massachusetts, U. S. A.). Analysis for calcium and magnesium ions was by atomic absorption spectrophotometry (AA/AE spectrophotometer 257, Instrumentation Laboratories). Plasma chloride ion concentrations were measured using a Corning Chloride

Analyser 925 (Corning, New York, U. S. A.). Total protein and albumin concentrations were measured using a Technicon® autoanalyser II (Technicon Instrument Company Limited, Basingstoke, U. K.) and globulin concentrations were calculated by subtracting the albumin concentration from the total protein concentration for each sample. The remainder of the analyses (phosphate ion, urea, creatinine, bilirubin, SAP, AST, ALT and GGT) were measured in a Cobas Mira Analyser (Roche Products Limited, Welwyn Garden City, U. K.). Values were compared to tables of normal ranges for the equine (Department of Veterinary Clinical Biochemistry, University of Glasgow Veterinary School; Kaneko, 1989).

2.19 Haematology

Haematological examinations were carried out using a Minos®Vet automatic cell counter (Minos, Montpellier, France). In addition an air-dried blood smear was stained with May Grunwald's Giemsa stain (The British Drug House Chemicals Limited, Poole, U. K.) and examined under a light microscope (SM Lux, Ernst Leitz GmBH, Wetzlar, Germany) for red and white blood cell morphology and differential white blood cell counts. Values were compared to tables of normal ranges for the equine (Department of Veterinary Pathology, University of Glasgow Veterinary School; Kaneko, 1989).

2.20 Bromsulphalein retention test of liver function

A sterile aqueous solution of 10% w/v bromsulphalein (sulphobromophthalein sodium salt, Sigma Chemical Company Limited, Poole, U. K.) was administered by intravenous bolus injection. Blood samples were taken into 10 ml lithium heparin syringes (Li-Heparin LH/10 Monovette®, Sarstedt Limited, Loughborough, U. K.) 6, 9 and 11 min after intravenous administration. The plasma concentration of bromsulphalein was analysed by absorbance spectrophotometry (Cecil Instruments Limited, Cambridge, U. K.) at a λ of 565 nm. The plasma concentrations were calculated from a plot of standard bromsulphalein concentrations (μg/ml) versus absorbance (Department of Veterinary Medicine, University of Glasgow Veterinary School). The elimination half-life of bromsulphalein in each animal was calculated from a plot of the bromsulphalein concentrations versus time (Cricket Graph version 1.3, Cricket software, Pennsylvania, U. S. A.).

2.21 Statistical analysis

All statistical analyses were performed using a standard statistical computer software package (Minitab Release 8 Macintosh® version, Clecom, Birmingham, U. K.). A non-parametric analysis of variance (Kruskal Wallis test) was used to compare pharmacokinetic parameters in horses, ponies and donkeys, and where statistically significant differences

were apparent, a Mann Whitney U test was used to compare the data between groups. The results of the analyses were considered to be significantly different when $p < 0.05$, highly significantly different when $p < 0.01$ and very highly significantly different when $p < 0.001$. Statistical analysis was not carried out, unless stated, on other data due to the low numbers of animals involved in the present study. In particular, the majority of studies were carried out in two ponies on two separate occasions. Also, there was a wide range in normal counts of viable bacteria and the development of clinical signs was considered more important than a statistically significant difference.

2.22 Pharmacokinetic analysis

The equations described in this section can be found in standard pharmacokinetic texts. The information used here was obtained from Baggot (1977a), Gibaldi (1991), Gibaldi and Perrier (1982), Rowland and Tozer (1989) and Sams (1987).

The disposition characteristics of each drug were calculated by analysing the changes in plasma drug concentrations versus time following a single intravenous bolus administration. All the pharmacokinetic analyses, of concentration versus time data for each animal on each occasion, were performed using a nonlinear regression Fortran IV curve stripping computer programme, CSTRIP (Sedman and Wagner, 1976). The programme generates up to 3 y-axis intercepts (A1, A2 and A3) and exponents (B1, B2 and B3) (last to first) for the equations best describing the data, and a linear correlation coefficient. The number of exponents best describing each data set was confirmed using Akaike's information criterion (Yamaoka *et al.*, 1978).

The y-axis intercepts and exponents for each data set were used to calculate the disposition kinetics of a drug in plasma. The elimination half-life or the time taken for 50% of the dose to be eliminated, in h or min, was estimated as 0.693/B1; where B1 is the hybrid rate constant for the terminal phase of disappearance of drug from the plasma. Similarly, the half-life of each distribution phase, in h or min, was calculated using 0.693/B2 and 0.693/B3. The initial plasma concentration (Cp0), in μg/ml, is the sum of the 0 time intercepts of the coefficients (A1, A2 and A3). The volume of the central compartment (Vc), in ml/kg, was calculated using the equation Vc = dose/Cp0, where the dose was in mg/kg bwt. The area under the zero moment curve or AUC, in μg.h/ml, was calculated by the sum of the ratio of each y-axis intercept and exponent, *i. e.* A3/B3 + A2/B2 + A1/B1. The area under the first moment curve (AUMC), *i. e.* the plasma concentration time versus time curve, in $\mu g.h^2/ml$, was calculated using the equation $AUMC = A3/(B3)^2 + A2/(B2)^2 + A1/(B1)^2$. The apparent volume of distribution (Vd_{area}) is the concentration of drug achieved after distribution is complete and is affected by both the dose and the extent of distribution of drug into the tissues. The Vd_{area}, in ml/kg, was calculated using the equation

Vd_{area} = dose/AUC.B1. The apparent volume of distribution at steady state (Vd_{SS}), in ml/kg, was calculated using the equation Vd_{SS} = dose.AUMC/$(AUC)^2$. The body clearance (CL_b), in ml/h.kg, was calculated using the equation CL_b = dose/AUC. The elimination rate constant (kel), in h, was calculated from the ratio of the CL_b to the Vc.

The area under the plasma concentration versus time curve for observed values (AUC_{obs}), in μg.h/ml, from time 0 to infinity was determined for observed concentrations using the trapezoidal rule. The area of the triangle between time 0 and the first sample was calculated by taking the average of the Cp0 and the first observed concentration multiplied by the first time point, and the area of the triangle between the last sample and 0 concentration was calculated by dividing the final concentration by the terminal slope, B1. The area under the first moment curve for observed values ($AUMC_{obs}$) from time 0 to infinity, *i. e.* the plasma concentration time versus time curve, in $\mu g.h^2/ml$, and the area of the triangle between time 0 and the first sample were calculated in a similar fashion. The area (AUMC) of the triangle between the last sample and concentration 0 was calculated using the product of time and the last plasma concentration divided by B1, plus the last concentration divided by B1 squared. The ratio of the AUMC to the AUC for any drug is a measure of its mean residence time (MRT), in h or min, which is a quantitative estimate of the persistence of a drug in the body and the time it takes for 63.2% of the dose to be eliminated.

A bi-exponential equation was related to a two-compartment model with first-order rate constants k12 and k21, in h or min, describing drug transfer between the compartments of the pharmacokinetic model. The rate constants were calculated using the equations B1 + B2 = k12 + k21 + kel and B1.B2 = k21.kel. A tri-exponential equation was related to a three-compartment model with rate constants k12, k21, k13 and k31 were calculated using the equations described by Gibaldi and Perrier (1982).

The exponents (B1 and B2), describing the plasma concentration versus time data following oral administration, were used to calculate the apparent half-lives (t1/2 B1 and t1/2 B2). Following oral drug administration, the lag time, in h or min, was calculated as the time between drug administration and the start of absorption, *i. e.* the point where extrapolated and residual curves intersected. An alteration in the rate of absorption produces changes in the time profile and plasma concentrations of a drug in the body however other factors (*e. g.* CL_b, apparent Vd, elimination half-life) remain constant. The availability (F), as a %, assuming CL_b remains constant, was calculated as the ratio of the total AUC_{obs} following oral administration to the AUC_{obs} following intravenous bolus administration, appropriately correcting for dose. The mean absorption time (MAT), in h or min, was calculated as the difference between the MRT following extravascular administration and the MRT following intravenous bolus administration.

2.23 Baseline SCFA concentrations *in vivo*

Samples of caecal liquor were taken at specific times (approximately 1030, 1330, 1730 and 2130 h), over two non-consecutive periods of 5 days from ponies I and II, prior to any drug administration. Samples were stored at -20 °C until analysis for SCFA concentrations. The results for each SCFA were expressed as a median (sample midpoint), lower and upper quartiles (Q1-Q3, range of 50% of the data points) and range (minimum to maximum), and, in addition, the VFA concentrations were expressed as a total, and the proportions of the main VFA (A, P and B) were expressed as a % of the total VFA concentrations (Table 2-20).

2.24 *In vitro* studies

An attempt was made to mimic the fate of the antimicrobial agents and the microbial fermentation patterns which appeared to occur in the gastrointestinal lumen *in vivo*.

Drug concentrations, bacteriological examinations and SCFA concentrations

Drug-free caecal liquor (pooled samples from animals I and II) was taken, on 4 separate occasions for each of the 4 antimicrobial drugs, and divided into aliquots in 15 ml glass tubes. In addition a known quantity of each drug (Table 2-21) and a magnetic stirring rod were added to each tube. The tubes were held in a wooden rack, covered with plastic film to prevent significant evaporation, and placed on a stirring plate (Corning hot plate PC101, Corning, New York, U. S. A.) in a MK3 Anaerobic Workstation (Don Whitley Scientific Limited, Shipley, U. K.) at 37 °C.

Changes in the number of viable bacteria were assessed for oxytetracycline only. Bacteriological examinations were carried out prior to incubation (no drug) and after 3 and 24 h incubation at concentrations of 0 and 80 μg/ml oxytetracycline on 4 separate occasions.

After 3 h incubation 3 ml of caecal liquor was removed from each tube and the incubation was continued for a total of 24 h. The destruction of antimicrobial drugs in caecal liquor was examined for by analysing the antimicrobial activity of the aliquots removed after 3 and 24 h incubation, using an agar gel diffusion assay immediately after collection. The remainder of each aliquot was stored at -20 °C until analysis for SCFA concentrations was performed. Allowance for the dilution of SCFA (by addition of drug standard) was made when calculating the SCFA concentrations.

Acid	n	Median	Q1-Q3	Range
SCFA concentration (mmol/l)				
L	57	**1.5**	0.0-4.8	**0.0-24.4**
A	57	**34.4**	28.5-39.3	**12.6-64.5**
P	57	**10.9**	8.5-14.6	**4.7-24.5**
IB	57	**0.0**	0.0	**0.0-2.9**
B	57	**12.2**	9.4-20.2	**4.8-67.3**
IV	57	**0.0**	0.0-1.2	**0.0-3.8**
V	57	**0.0**	0.0	**0.0-9.2**
Total VFA	57	**65.0**	52.3-78.2	**24.4-109.2**
VFA concentration (%)				
A	57	**53.1**	48.9-58.9	**27.9-67.0**
P	57	**18.7**	13.8-24.4	**4.9-38.5**
B	57	**21.3**	16.9-32.4	**8.7-66.7**
P+B	57	**43.3**	37.1-50.0	**27.1-72.1**

Table 2-20. Baseline values of SCFA in equine caecal liquor

Concentration (µg/ml)	Standard (volume and concentration)	Caecal liquor (ml)
0	0	10.00
0.25	12.5 µl of 200 µg/ml	9.99
1	50 µl of 200 µg/ml	9.95
5	250 µl of 200 µg/ml	9.75
10	500 µl of 200 µg/ml	9.50
20	1000 µl of 200 µg/ml	9.00
40	2000 µl of 200 µg/ml	8.00
80	4000 µl of 200 µg/ml	6.00

Table 2-21. Standard antimicrobial agent concentrations for studies in equine caecal liquor *in vitro*

Acid pH

Antimicrobial agents were incubated in chloride buffer (0.02 M, pH 1.9) to mimic the effects of the equine gastric pH (pH 1.63-1.97, Sangiah *et al.*, 1989) on antimicrobial activity. A series of standard concentrations of each drug were made from a 10 mg/ml standard solution (0.01 g standard compound dissolved in 10 ml stock sterile buffer solution) (Table 2-22). The acid samples were mixed for approximately 20 s using a Griffin vortex shaker/stirrer (Griffin and George Limited, Loughborough, U. K.) and samples were incubated at room temperature (20 °C) for 1 h. An aliquot of 100 µl of each sample was diluted with 900 µl of the appropriate stock sterile buffer solution and mixed for approximately 20 s using a Griffin vortex shaker/stirrer. The standard samples were analysed immediately after incubation by agar gel diffusion assay. The experiment was repeated on 4 occasions for each drug.

Binding to hay

The binding of antimicrobial agents to hay was examined by incubating a series of standard drug concentrations with hay at a pH similar to that found in the equine stomach (pH 1.63-1.97, Sangiah et al., 1989) and small intestine (pH 6.32-7.47, Mackie and Wilkins, 1988). A sample of hay was cut into lengths of approximately 10 mm and 1 g was added to a series of 50 ml screw cap plastic tubes (Greiner labortechnik Limited, Dursley, U. K.) containing phosphate buffer (0.05 M, pH 7.0) and standard concentrations of the antimicrobial agent (Table 2-22). Control tubes of standard drug concentrations without hay were incubated alongside the test tubes on a rotary mixer (Eschmann Brothers and Walsh Limited, Shoreham-By-Sea, U. K.) at 37 °C in a hot room for 3 h. Following incubation the concentrations of the antimicrobial agent in the supernatant of the tubes containing hay were compared with control concentrations, by agar gel diffusion assay. The experiment was carried out on one occasion for penicillin G, six occasions for ampicillin and one occasion for amikacin.

Stability of 7 SCFA in equine caecal liquor *in vitro*

Stability of SCFAs in equine caecal liquor was assessed by the repeated analysis of a series of samples of pooled caecal liquor. A fresh pooled sample of caecal liquor was divided into a series aliquots in glass universal bottles which were subjected to a variety of different conditions. One set of four aliquots was analysed on the same day for SCFA concentrations; one set of aliquots was sterilized by radiation (50 kiloGray in 40 h, ^{60}Co source, Scottish Universities Reactor and Research Centre, East Kilbride, U. K.); one set of aliquots was stored at room temperature (20 °C); one set at 4 °C; and one set at -20 °C. After 2 days,

Concentration (µg/ml)	Standard (volume and concentration)	Buffer volume (ml)
0	0	10.00
10	10 µl of 10 mg/ml	9.99
20	25 µl of 10 mg/ml	9.98
50	50 µl of 10 mg/ml	9.95
100	100 µl of 10 mg/ml	9.90

Table 2-22. Standard antimicrobial drug concentrations for studies at acid (pH 1.9) and neutral (pH 7.0) pH

SCFA concentrations were analysed in all 16 samples. The irradiated and 20 °C samples had magnetic stirring rods added and were placed on a stirring plate (Corning hot plate PC101, Corning, New York, U. S. A.) in a MK3 Anaerobic Workstation (Don Whitley Scientific Limited, Shipley, U. K.) at 37 °C for 24 h. The SCFA concentrations were measured after 24 h incubation. In addition, the SCFA concentrations were measured after 14 days storage of samples at 4 and -20 °C.

A bacteriological examination was carried out following irradiation of one set of aliquots. A volume of 1 ml of each aliquot was inoculated onto a 7% sheep blood (210101, Becton-Dickinson, Dublin, Ireland) agar plate (CM271, Oxoid, Unipath, Basingstoke, U. K.) for the detection of coliforms, a 7% horse blood (SR50, Oxoid, Unipath) agar plate (CM271, Oxoid, Unipath) for the detection of *Bacteroides spp.* and MRS agar (CM361, Oxoid, Unipath) for detection of lactobacilli. After suitable incubation, there was only 1 sheep blood plate with a single coliform colony on it (which may have been a contaminant), therefore the samples were considered to be bacteria free following the irradiation procedure.

SCFA concentrations following storage were compared with initial SCFA concentrations using a Mann Whitney U test. There were no statistically significant changes in lactic, isobutyric, isovaleric or valeric acid concentrations following incubation, irradiation or storage. There was a statistically significant decrease in the concentration of acetic acid (median) from 28.8 mmol/l to 19.5 mmol/l and 19.6 mmol/l, respectively, following irradiation plus incubation and after 14 days at 4 °C ($p < 0.05$). There was a statistically significant increase in the concentration of propionic acid from 8.2 mmol/l to 10.8 mmol/l after 2 days storage at 20 °C and a decrease to 6.5 mmol/l after 14 days storage at 4°C ($p < 0.05$). There was a statistically significant increase in the median concentration of butyric acid from 5.7 mmol/l to 14.6 mmol/l after irradiation plus incubation and a decrease to 2.9 mmol/l after 14 days storage at 4 °C ($p < 0.05$). Thus, SCFA concentrations were considered to be stable if stored at 4° C for 2 days and at -20 °C for 14 days, *i. e.* in the manner that unknown samples were treated. There were few alterations in SCFA concentrations following irradiation *i. e.* in the absence of bacteria.

2.25 Caecal volume

The volume of caecal contents was measured in 12 ponies at slaughter. After each animal was euthanised, the caecum was tied off and removed from the small intestine and colon. The contents of the caecum were emptied into a graduated plastic bucket and the volume (l) estimated. A computer programme (Cricket graph version 1.3, Cricket software, Pennsylvania, U. S. A.) was used to plot the volume of the caecal contents (l) versus the bwt (kg) and to calculate the line of best fit and r (Figure 2-14). The relationship between

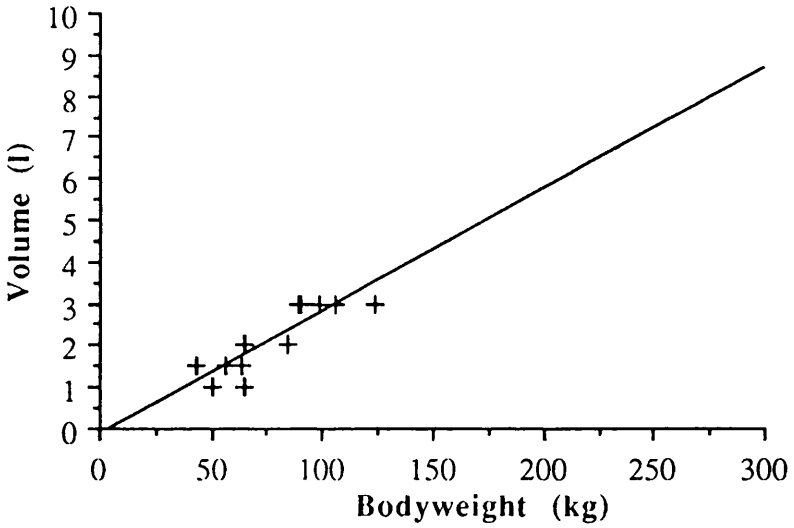

Figure 2-14. The relationship between caecal volume and bodyweight in ponies

caecal volume (l) and bwt (kg) was reasonably linear (r = 0.748), and the line of best fit, y = -0.157 + 0.0293x, was used to calculate the approximate caecal volume in live animals. Two of the ponies with caecal fistulas (I and II) were weighed on 12 occasions over a period of 11 months. The bwt (mean±SD) for each pony was 233±16 and 216±14 kg and the calculated caecal volume (mean±SD) was 6.67±0.47 and 6.17±0.40 l, respectively. The volume of caecal contents in pony III (260 kg bwt) was estimated as 7.46 l.

3 Studies with penicillin G

3.1 Introduction

The penicillins remain the most popular group of antimicrobial agents used in equine practice due to the suitable bacterial susceptibilities*in vitro*, good general tolerance, satisfactory clinical responses and cost (Ricketts and Hopes, 1984). Procaine penicillin is used widely to treat equine infections caused by Gram positive bacteria. However, there are a number of factors which diminish the usefulness of penicillin G (benzylpenicillin). Penicillin G is inactivated rapidly in the gastrointestinal tract, by ß-lactamase enzymes, by protein binding and by acid (De Louvois and Hurley, 1977, Prins, 1987). The destruction of penicillin G by gastric acid means that it must be administered parenterally to attain suitable therapeutic plasma or serum concentrations.

Plasma and serum concentrations of penicillin G have been reported following intravenous and intramuscular administration to horses at a variety of different dose rates. The pharmacokinetics of sodium penicillin G were studied by Dürr (1976) following intravenous administration at dose rates of 21 and 36.3 mg/kg bwt (35,000 or 60,500 iu/kg bwt). Knight (1975) suggested that in the horse dose rates of 12.5, 18 or 37.6 mg/kg (20,000, 30,000 or 60,000 iu/kg) bwt, similar to those used in man, were appropriate for the intravenous administration of penicillin G potassium. Prescott and Baggot (1985) and Baggot and Prescott (1987) recommended a regime of either intravenous or intramuscular administration of penicillin G sodium at the lower dose rate of 9-12 mg/kg (15,000-20,000 iu/kg) bwt four times daily.

The procaine salt of penicillin G has been administered to horses at dose rates of 9-17.5 mg/kg bwt by the intramuscular route (Brown *et al.*, 1984a, Knight, 1975, Stover *et al.*, 1981, Prescott and Baggot, 1985). Administration of procaine penicillin G at a dose rate of 17.5 mg/kg bwt every 12-24 h has been recommended for clinical use (Prescott and Baggot, 1985).

Penicillin V (phenoxymethylpenicillin) is stable in the presence of gastric acid. Consequently, penicillin V should have a higher systemic availability than penicillin G following oral administration, and should produce suitable therapeutic plasma or serum concentrations, and provide an alternative for long-term penicillin therapy. Schwark *et al.* (1983) examined the absorption and distribution patterns of penicillin V potassium following oral administration to horses at a dose rate of 73 mg/kg (110,000 iu/kg) bwt. Similarly, Ducharme *et al.* (1983) reported that oral administration of penicillin V at a dose rate of 44 or 73 mg/kg (66,000 or 110,000 iu/kg) would provide a suitable alternative to parenteral

administration of penicillin G. However, Baggot *et al.* (1990) reported that the systemic availability of penicillin V was low in adult horses compared with foals (1.65% and 16.04%, respectively), and that it was lower than the systemic availability of penicillin G in adult horses (2.87%).

There have been reports of adverse reactions occurring in horses following the administration of the penicillins but host toxicity has not limited their use (Prescott and Baggot, 1985). Roberts and English (1979) reviewed adverse reactions to antimicrobial agents and noted that hypersensitivity was by far the most common adverse reaction to the penicillins. This type of reaction, along with muscle tenderness and localised oedema, was much more common following intramuscular administration compared with intravenous administration of the penicillins (Aronson and Brownie, 1978). Nielsen *et al.* (1988) reported 11 cases of acute procaine toxicity following intramuscular administration of penicillin G. In addition, the use of procaine salts has to be avoided in competition animals. Penicillin-induced anaemia (Step *et al.*, 1991) is rare in the horse and in man but in the horse the percentage of individuals with anti-penicillin antibodies is high (Blue *et al.*, 1987). Baggot *et al.* (1990) reported that five out of six horses developed clinical signs of lethargy, increased heart rate, increased respiratory rate and abdominal pain approximately 6 h after oral administration of penicillin V or penicillin G at a dose rate of 90 mg/kg bwt.

The purpose of the present study was to determine the plasma disposition and pharmacokinetics of penicillin G sodium following intravenous administration at the dose rate of 10 mg/kg bwt to horses, ponies and donkeys. Alterations in the gastrointestinal microflora were studied by bacteriological examination of serial faecal samples and large intestinal fermentation was studied using faecal SCFA concentrations. Faecal dry matter content and faecal consistency were used as an indicator of the presence or absence of diarrhoea. Similar studies were carried out in ponies with cannulated caecal fistulas following a single intravenous or oral administration of penicillin G at a dose rate of 10 mg/kg bwt. In addition, drug and bacteriological analyses were carried out on caecal liquor, and plasma biochemistry and haematology were monitored.

3.2 Materials and Methods

3.2.1 Intravenous administration of penicillin G to horses, ponies and donkeys

Three thoroughbred geldings (No. 3-5), 3 ponies (No. 7, 8 and 10) and three donkeys (No. 14-16) were used as outlined in the general Materials and Methods. Penicillin G sodium was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples were taken prior to drug administration and at 0.033, 0.083, 0.25, 0.5 0.75, 1, 1.5, 2, 4, 6,

8, 12 and 24 h, except in horse 3 there was a 0.33 h sample not a 0.25 h sample, and in horse 4 there was a 0.1 h sample not a 0.083 h sample, and in donkey 14 there was a 0.117 h sample and no 0.033 or 0.083 h samples. Faecal samples were taken at 0, 24, and 48 h after drug administration for drug analysis, bacteriological examination, SCFA analysis and the measurement of dry matter content.

3.2.2 Intravenous administration of penicillin G to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 2 occasions (1 and 2). Penicillin G sodium was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis, measurement of pH and SCFA analysis were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, with additional plasma samples at 0.033 and 0.083 h, and an additional caecal liquor sample at 48 h. Bacteriological examination of caecal liquor was carried out at 0, 24 and 48 h. Faecal samples were taken for drug analysis, SCFA analysis and the measurement of dry matter content at 0, 24 and 48 h. Plasma biochemistry and haematological examinations were carried out at 0, 24 and 48 h.

3.2.3 Oral administration of penicillin G to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 2 occasions (1 and 2) at least 6 days after intravenous administration of penicillin G sodium. Penicillin G sodium was administered *via* nasogastric tube at a dose rate of 10 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis, pH measurement and SCFA analysis were taken at 0, 0.25, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, with further caecal liquor samples taken at 28, 32, 48, 52, 56, 72, 96 and 168 h. Bacteriological examinations of caecal liquor were carried out at 0, 24, 48, 72, 96 and 168 h. Faecal samples were taken for the measurement of drug concentrations, SCFA concentrations and dry matter content at 0, 24, 48, 72, 96 and 168 h. Samples were taken for plasma biochemistry and for haematological examinations at 0, 24, 48 on occasions 1 and 2, and at 168 h on occasion 2.

3.2.4 *In vitro* studies with penicillin G

A range of concentrations of penicillin G sodium were incubated for 3 and 24 h in caecal liquor at body temperature in an anaerobic environment, and drug and SCFA concentrations were measured following incubation as described in the general Materials and Methods. A

range of concentrations of penicillin G sodium were incubated at acid pH for 1 h at room temperature as outlined in the general Materials and Methods. A range of concentrations of penicillin G sodium were incubated in the presence of chopped hay at body temperature for 3 h at gastric and small intestinal pH as outlined in the general Materials and Methods.

3.3 Results of intravenous administration of penicillin G to horses, ponies and donkeys

3.3.1 Plasma disposition and pharmacokinetics

A semilogarithmic plot of plasma concentrations (mean±SEM), following intravenous administration of penicillin G to horses, ponies and donkeys, is shown in Figure 3-1. Individual and mean plasma concentrations are given in Appendix A (Tables A1-A3). In all 3 groups, the initial plasma concentration versus time plots were similar, however the decline phase of the plasma concentration versus time plot was less steep in horses than it was in ponies and donkeys. Penicillin G was detected in plasma (>0.01 μg/ml) for up to 8 h in horses, and 6 h in ponies and donkeys.

The pharmacokinetic parameters calculated from the bi-exponential equations used to describe the plasma concentration versus time data are given in Table 3-1 and in Appendix A (Tables A4-A6). The harmonic mean of the elimination half-life calculated for horses, ponies and donkeys was similar and quite short. The elimination half-life was longest in horses followed by donkeys and then ponies, whereas the MRT was longest in horses, followed by ponies and then donkeys. There were no statistically significant differences, calculated using a Kruskal Wallis test, between the pharmacokinetic parameters calculated for the three groups. The CLb was very high (*circa* 500 ml/h.kg).

3.3.2 Faecal concentrations

No penicillin G was detected in faeces following intravenous administration to horses, ponies and donkeys.

3.3.3 Bacteriological examinations

Counts of viable bacteria in faeces (mean±SEM) following intravenous administration of penicillin G are shown in Figures 3-2, 3-3 and 3-4, and the individual and mean data are given in Appendix A (Tables A7-A9).

Salmonella spp. and *C. difficile* were selected for but not isolated.

In the 3 horses there were no marked changes in the number of coliforms, *Bacteroides spp.* or *Clostridium spp.* isolated (Figure 3-2). There was an apparent reduction in the mean

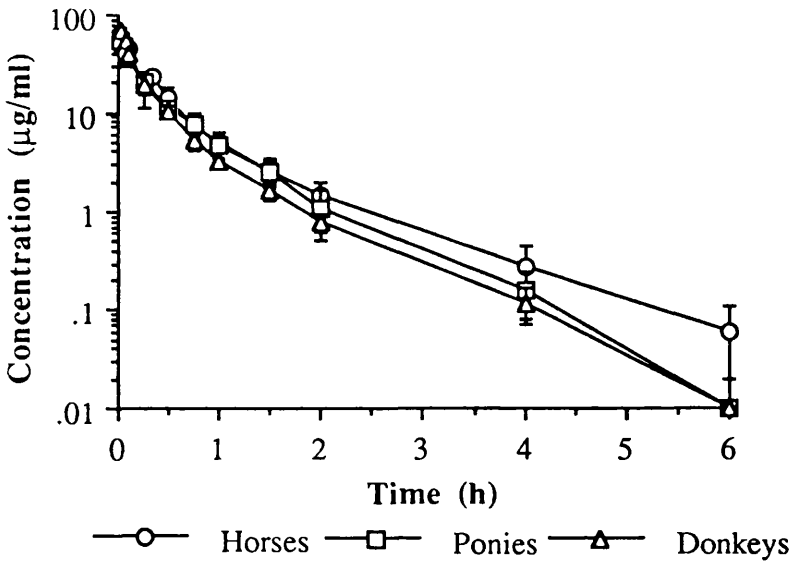

Figure 3-1 Plasma concentrations (mean±SEM) of penicillin G following intravenous administration to horses, ponies and donkeys

Parameter	Horses (n=3)	Ponies (n=3)	Donkeys (n=3)
t1/2 B2 (min)*	5.85	2.52	4.28
t1/2 B1 (min)*	38.95	27.25	31.52
Cp0 (µg/ml)	62.90±8.87	74.52±14.65	126.42±43.13
Vc (ml/kg)	165.98±25.01	145.14±28.38	95.91±24.75
AUC_{obs} (µg.h/ml)	23.03±5.07	21.19±2.00	23.66±2.51
$AUMC_{obs}$ (µg.h^2/ml)	15.07±4.90	12.00±2.96	9.79±1.66
AUC (µg.h/ml)	22.41±5.35	20.15±1.95	21.91±0.55
AUMC (µg.h^2/ml)	16.53±5.86	12.24±2.77	9.90±1.40
MRT (min)	38.07±5.29	34.35±8.94	24.46±1.83
Vd_{area} (ml/kg)	537.25±205.21	368.42±86.87	357.25±30.67
Vd_{ss} (ml/kg)	362.32±106.47	314.05±91.85	204.53±3.00
CL_b (ml/h.kg)	514.46±145.60	505.36±47.46	462.86±40.04
k_{el} (/h)	3.03±0.49	3.72±0.73	5.71±1.78
k_{21} (/h)	2.62±0.83	6.51±2.07	2.26±0.32
k_{12} (/h)	2.52±0.40	7.72±3.81	3.14±1.40

Table 3-1. Disposition kinetics of penicillin G in plasma following intravenous administration to horses, ponies and donkeys

Key: data as mean ±SEM; * harmonic mean

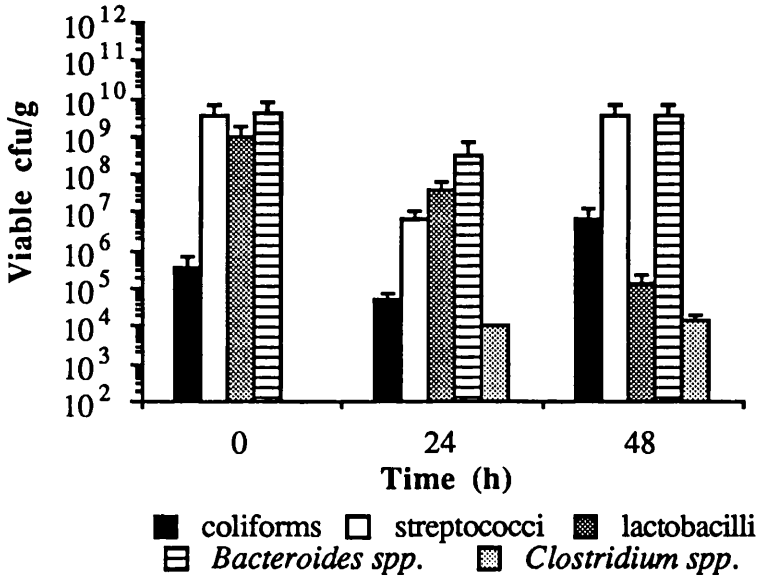

Figure 3-2. Counts of viable bacteria in faeces (mean±SEM) following intravenous administration of penicillin G to horses

number of streptococci and lactobacilli isolated at 24 h after drug administration, but this appears to have been due to high initial counts of streptococci in horses 3 and 5 (10^9-10^{10}/g), and lactobacilli in horse 4 (10^9/g).

In ponies, there was an increase in the mean number of coliforms, up to 10^9/g, isolated at 24 and 48 h after drug administration (Figure 3-3). There was an increase in the viable number of coliforms isolated from all 3 ponies but it was most marked in ponies 8 and 10 (10^{10}/g). In the 3 ponies there were no marked changes in the number of streptococci, lactobacilli or *Clostridium spp.* isolated. There was an apparent increase in the mean number of *Bacteroides spp.* (10^9/g) isolated at 24 h after intravenous administration of penicillin G to ponies. This was due to the isolation of a low number of *Bacteroides spp.* (10^5-10^8/g) from all samples, except at 24 h after drug administration to pony 10 (10^{10}/g). The low numbers of Bacteroides spp. isolated may have been due to exposure of the organisms to oxygen (air). There was a relatively high number of *Clostridium spp.* (10^6/g) isolated from pony 10 prior to drug administration.

There was a slight increase in the mean number of coliforms isolated from donkey faeces at 48 h after penicillin G administration (Figure 3-4). This was due to an increase in the number of coliforms (10^8/g) isolated from donkey 14 at 48 h after drug administration. There were no marked changes in the mean number of streptococci, lactobacilli or *Bacteroides spp.* isolated. The mean number of *Clostridium spp.* isolated at 48 h after drug administration was high (10^7/g) due to high numbers of *Clostridium spp.* isolated from donkeys 14 and 15 (10^6-10^8/g) at this time. The numbers of *Bacteroides spp.* isolated from donkeys faeces was low (10^5-10^8/g) throughout the study.

Lactobacilli identified using the API system were *L. acidophilus* and *L. fermentum*. *Bacteroides spp.* identified using the API system were *B. asaccharolyticus*, *B. eggerthii*, *B. ovatus*, and *B. thetaiotaomicron*. *Clostridium spp.* identified using the API system were *C. butyricum*, *C. clostridiiforme* and *C. tertium*. Other bacteria identified using the API system were *Actinomyces israelii*, *Actinomyces meyeri*, *Bifidobacter adolescentis*, *Eubacterium lentum*, *Peptococcus spp.* and *Streptococcus spp.*.

3.3.4 Faecal SCFA concentrations

Faecal lactic acid and total VFA concentrations (mean ±SEM), following intravenous administration of penicillin G to horses, ponies and donkeys are shown in Figures 3-5 and 3-6, and the individual and mean data are given in Appendix A (Tables A10-A19).

The faecal SCFA concentrations in horses, ponies and donkeys were very variable following the intravenous administration of penicillin G. Lactic acid concentrations in faeces remained

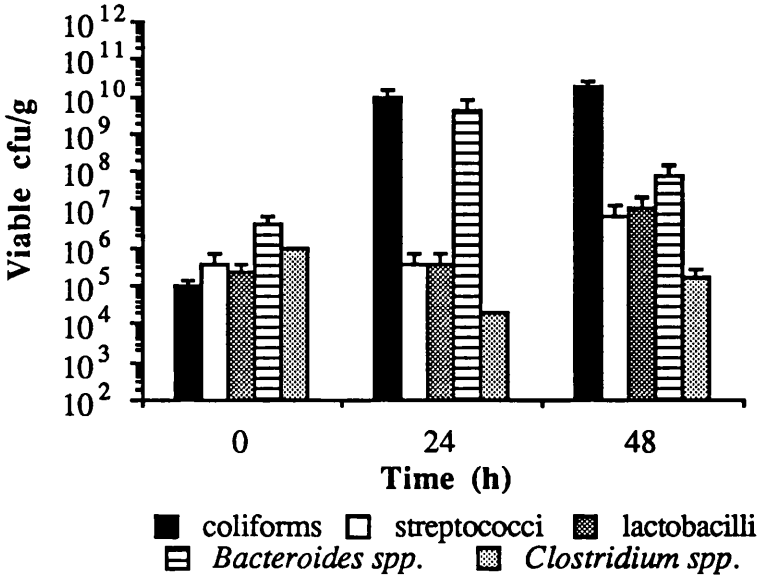

Figure 3-3. Counts of viable bacteria in faeces (mean±SEM) following intravenous administration of penicillin G to ponies

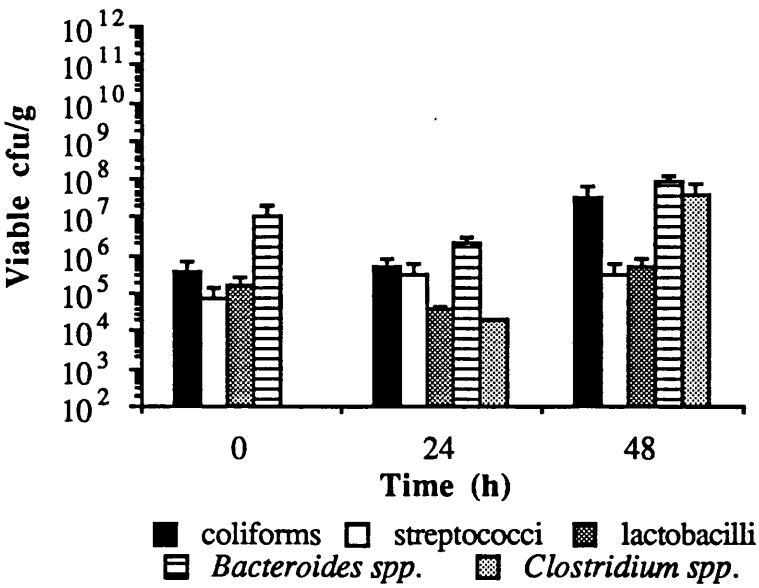

Figure 3-4. Counts of viable bacteria in faeces (mean±SEM) following intravenous administration of penicillin G to donkeys

low following intravenous administration of penicillin G to horses, ponies and donkeys and were all within the normal range of 0.0-24.4 mmol/l (Figure 3-5). In horses, ponies and donkeys, mean total VFA concentrations were within the normal range of 24.4-109.2 mmol/l at 0, 24 and 48 h after intravenous administration of penicillin G (Figure 3-6). In individual horses, ponies and donkeys, there were considerable fluctuations in the total and individual VFA concentrations. However, there did not appear to be any trend in the observed concentrations that was associated with antimicrobial administration.

3.3.5 Faecal dry matter content and consistency

A plot of faecal dry matter content (mean±SEM) following intravenous administration of penicillin G to horses, ponies and donkeys is shown in Figure 3-7, and individual and mean data are given in Appendix A (Tables A20-A22).

There were no marked alterations in the mean faecal dry matter content following intravenous administration of penicillin to horses and donkeys. There was a reduction in the faecal dry matter content at 24 h in horse 4 (15.44%). In ponies, the mean faecal dry matter content was reduced at 48 h after drug administration (<17%) and this reflected a reduction in the faecal dry matter content in ponies 7 and 8 (15.86 and 14.98%, respectively). In addition, there appeared to be an increase in the faecal dry matter content in donkey 15 at 24 and 48 h after intravenous administration of penicillin G (>30%).

No alterations in faecal consistency were observed following intravenous administration of penicillin G to horses, ponies and donkeys.

3.4 Results of intravenous administration of penicillin G to ponies with cannulated caecal fistulas

3.4.1 Plasma disposition and pharmacokinetics

The plasma concentrations of penicillin G following intravenous administration to ponies I and II on occasions 1 and 2 are given in Appendix A (Table A23).

The plasma concentration versus time data from pony I1 could not be described using a bi-exponential equation, therefore the parameters were calculated from a mono-exponential equation. A bi-exponential equation best described the data from ponies I2, II1 and II2. The pharmacokinetic parameters calculated from the plasma concentration versus time data of each animal on each occasion are given in Table 3-2. The pharmacokinetic parameters that were calculated for ponies I2 and II1 were similar to those obtained from ponies 7, 8 and 10 in the previous study (3.3.1). However, the elimination half-life and MRT calculated for

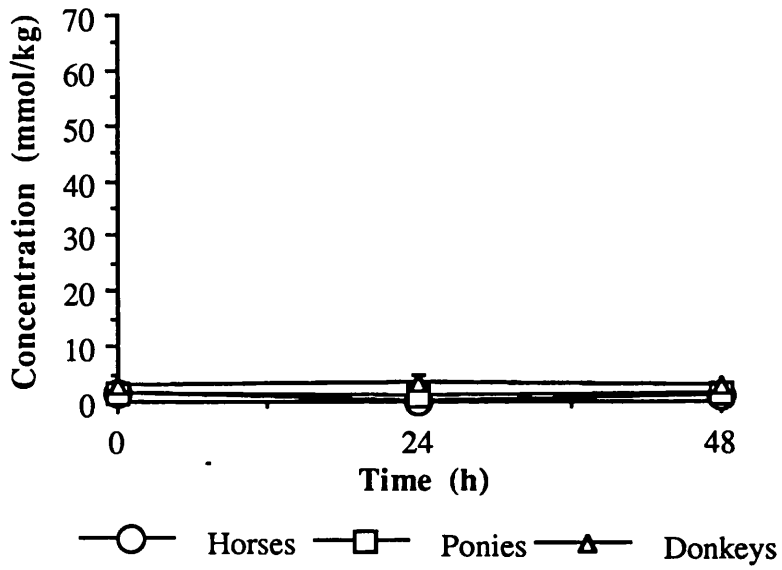

Figure 3-5. Lactic acid concentrations (mean±SEM) in faeces following intravenous administration of penicillin G to horses, ponies and donkeys

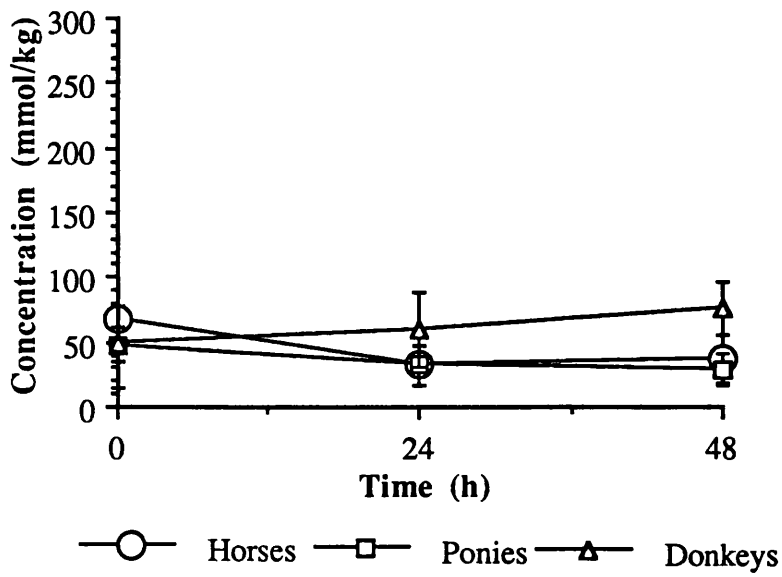

Figure 3-6. Total VFA concentrations (mean±SEM) in faeces following intravenous administration of penicillin G to horses, ponies and donkeys

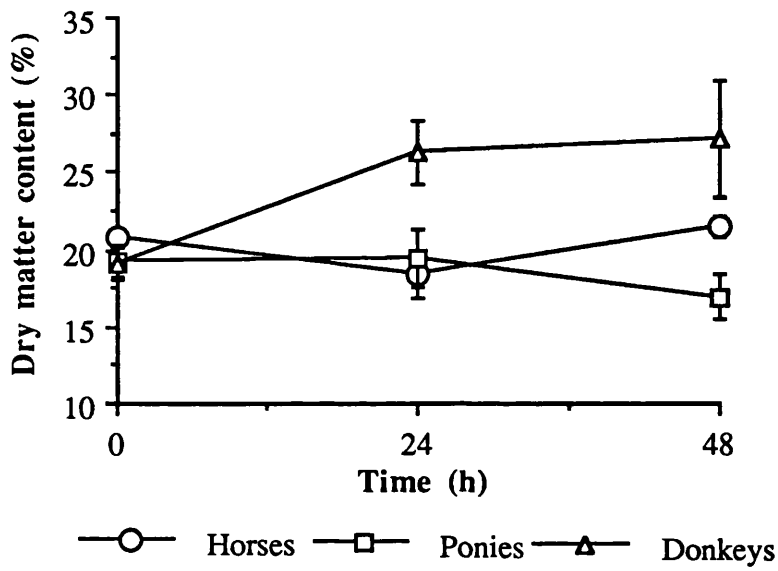

Figure 3-7. Faecal dry matter content (mean±SEM) following intravenous administration of penicillin G to horses, ponies and donkeys

Parameter	I1*	I2§	II1§	II2§
t1/2 B2 (min)	-	1.32	7.26	2.40
t1/2 B1 (min)	69.60	38.82	36.78	62.70
Cp0 (μg/ml)	15.86	132.50	59.43	32.40
Vc (ml/kg)	630.36	75.47	168.26	308.65
AUC_{obs} (μg.h/ml)	29.13	22.19	21.29	20.52
$AUMC_{obs}$ (μg.h^2/ml)	49.88	16.49	11.97	26.57
AUC (μg.h/ml)	26.53	20.32	20.76	19.82
AUMC (μg.h^2/ml)	44.36	15.76	12.79	28.21
MRT (min)	102.74	44.59	33.73	77.69
Vd_{area} (ml/kg)	630.36	459.82	425.77	760.63
Vd_{SS} (ml/kg)	630.36	381.70	296.79	717.94
CL_b (ml/h.kg)	376.98	492.15	481.71	504.53
k_{el} (/h)	0.60	6.52	2.86	1.64
k_{21} (/h)	-	5.27	2.26	7.02
k_{12} (/h)	-	21.37	1.73	9.31

Table 3-2. Disposition kinetics of penicillin G in plasma following intravenous administration to ponies

Key: * calculated from a mono-exponential equation; § calculated from a bi-exponential equation

ponies I1 and II2 were very long.

3.4.2 Caecal liquor and faecal concentrations

The caecal concentrations of penicillin G following intravenous administration to ponies I and II on occasions 1 and 2 are given in Appendix A (Table A24). No penicillin G was detected in the caecal liquor samples from ponies I1, II1 or II2 following intravenous administration. The caecal liquor concentrations of penicillin G in pony I2 are shown in Figure 3-8. A maximum caecal liquor concentration of 0.60 μg/ml was measured at 0.5 h after drug administration.

Drug disposition within the caecum was described using AUC and AUMC for observed values and the ratio of these (MRT). The AUC was 0.77 μg.h/ml, the AUMC was 0.93 $\mu g.h^2/ml$ and the MRT was 72.40 min.

There was no penicillin G detected in the faecal samples from the ponies following intravenous administration.

3.4.3 Bacteriological examinations

Counts of viable bacteria in caecal liquor following intravenous administration of penicillin G are shown in Figures 3-9 to 3-13, and the individual data is given in Appendix A (Table A25).

Salmonella spp. and *C. difficile* were selected for but not isolated.

There were no marked changes in the number of coliforms isolated from ponies I1, II1 and II2 (Figure 3-9). The number of coliforms isolated from pony I2 was high (10^8/ml) prior to drug administration and fell to a normal value of 10^3-10^7/ml at 24 and 48 h after drug administration.

There were no marked alterations in the number of streptococci isolated following intravenous administration of penicillin G to ponies I1, I2, II1 and II2 (Figure 3-10). The number of streptococci isolated from pony II1 increased to 10^8/ml at 24 h after intravenous administration of penicillin G.

There were no marked alterations in the number of viable lactobacilli isolated following the intravenous administration of penicillin G to ponies I1, I2, II1 and II2 (Figure 3-11). Lactobacilli identified using the API system were *L. acidophilus* and *L. jensenii*.

There were no marked alterations in the number of *Bacteroides spp.* isolated following intravenous administration of penicillin G to ponies I1, I2, II1 and II2 (Figure 3-12).

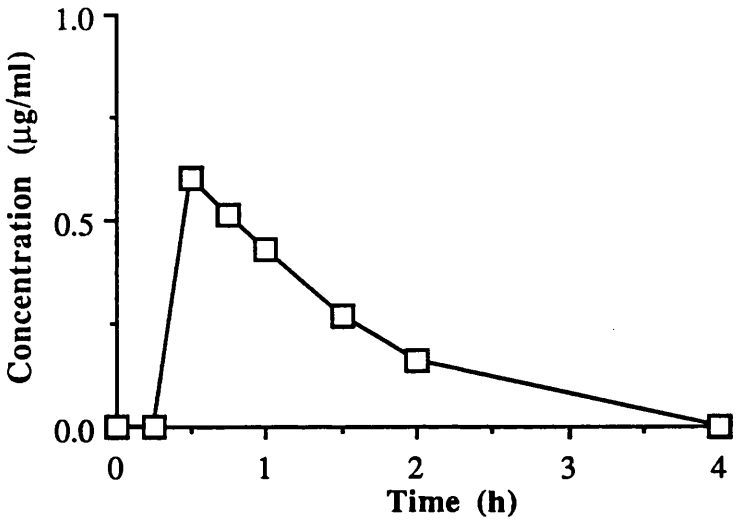

Figure 3-8. Caecal liquor concentrations of penicillin G following intravenous administration to pony I2

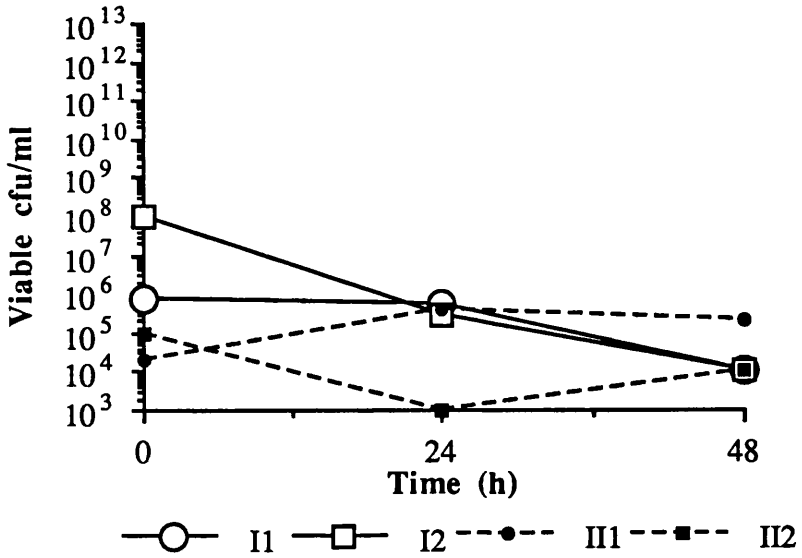

Figure 3-9. Counts of viable coliforms in caecal liquor following intravenous administration of penicillin G to ponies

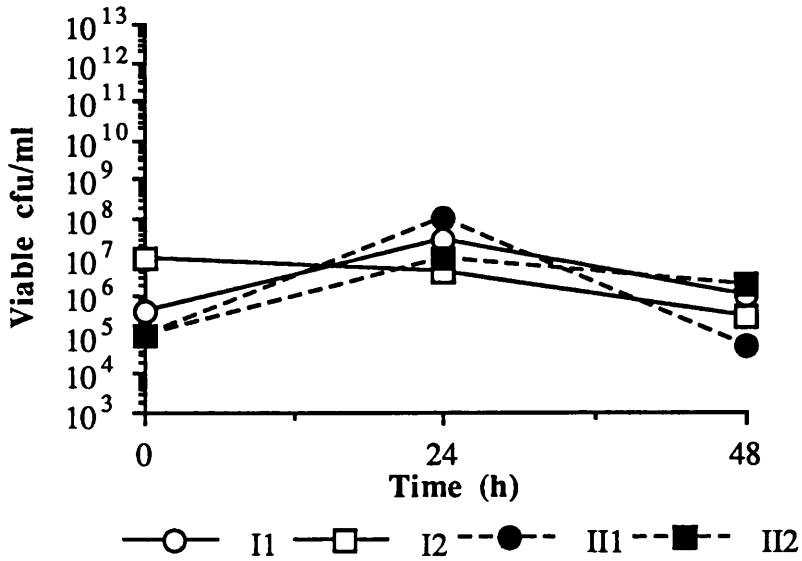

Figure 3-10. Counts of viable streptococci in caecal liquor following intravenous administration of penicillin G to ponies

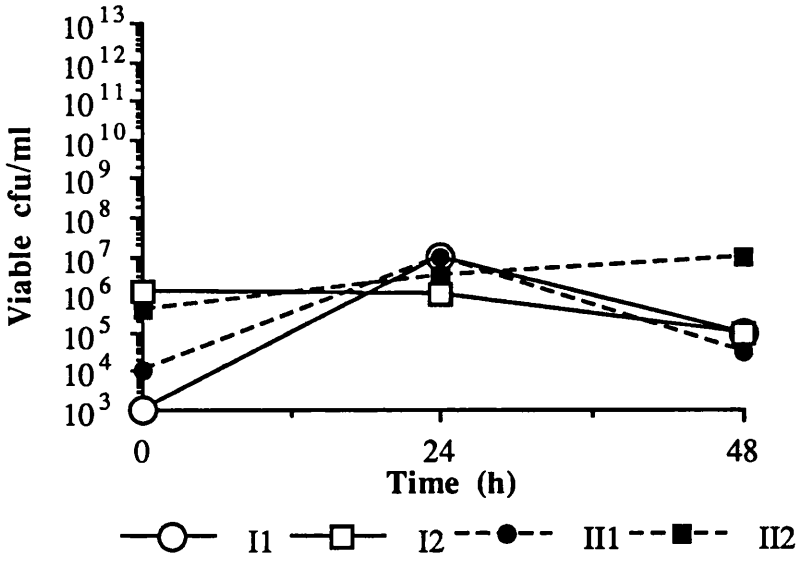

Figure 3-11. Counts of viable lactobacilli in caecal liquor following intravenous administration of penicillin G to ponies

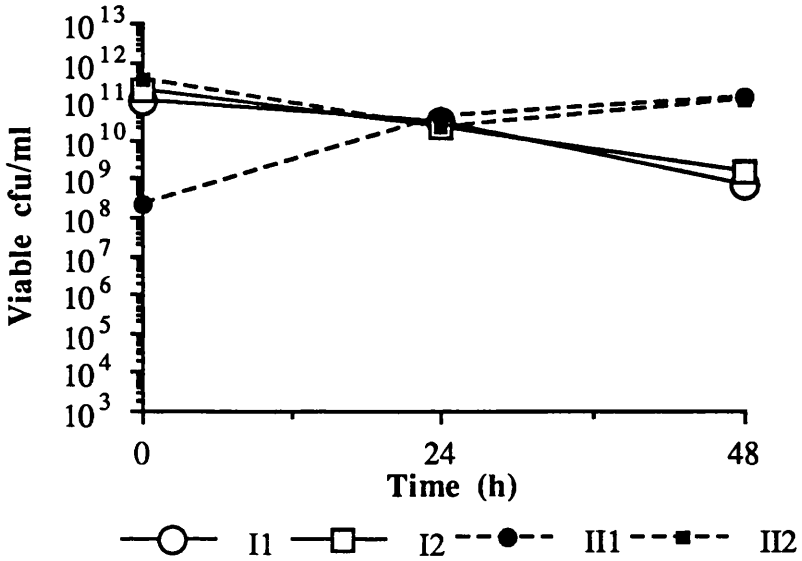

Figure 3-12. Counts of viable *Bacteroides spp.* in caecal liquor following intravenous administration of penicillin G to ponies

However, the number of *Bacteroides spp.* isolated was low (10^8/ml) prior to drug administration to pony II1, and at 48 h after drug administration to pony I1. *Bacteroides spp.* identified using the API system were *B. capillosus*, *B. oralis* and *B. uniformis*.

There were no marked alterations in the number of *Clostridium spp.* (Figure 3-13) isolated from any of the ponies at any of the sampling times prior to or following intravenous administration of penicillin G. However, there were quite high numbers of *Clostridium spp.* (10^6/ml) isolated from ponies I1 and I2 prior to drug administration, and from pony I2 at 24 h after drug administration. *Clostridium spp.* identified using the API system were *C. clostridiiforme* and *C. ramosum*.

Other bacteria identified using the API system were *Eubacterium lentum*, *Actinomyces naeslundii*, *Peptococcus spp.* and *Streptococcus spp.*.

3.4.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

Caecal liquor pH values following intravenous administration of penicillin G are shown in Figure 3-14, and the individual data is given in Appendix A (Table A26). The caecal pH was slightly high (7.4) at 0.25 h after drug administration to pony I1. In ponies I2 and II2 the caecal liquor pH was slightly lower than normal (<6.8) at 0.75, 1, 4 and 8 h and at 0, 0.25, 0.75, 2, 4, and 8 h, respectively, following intravenous administration of penicillin G. At other sampling times the pH of caecal liquor was normal (6.8-7.2).

Caecal liquor lactic acid concentrations, following intravenous administration of penicillin G to ponies are shown in Figure 3-15, and the individual data is given in Appendix A (Tables A27a-A30a). In pony I1, lactic acid concentrations were increased to 7.1-10.1 mmol/l at 0.5, 0.75 and 1 h, but these concentrations were all within the normal concentration range of 0.0-24.4 mmol/l. In pony I2, lactic acid concentrations were increased to 6.2-59.5 mmol/l at 0.5, 1.5, 2, 4, 6, 8, 12 and 48 h after drug administration, and were outside the normal range at 4, 6 and 8 h after drug administration. However, in ponies II1 and II2, lactic acid concentrations in caecal liquor did not increase and were within the normal range of 0.0-24.4 mmol/l at all times sampled.

Caecal liquor total VFA concentrations are shown in Figure 3-16, and total VFA and individual acid concentrations are given in Appendix A (Tables A27a-A30a). There were fluctuations in total and individual VFA concentrations about the normal ranges. Prior to drug administration to pony I1, the propionic acid concentration of 3.0 mmol/l was lower than the normal range of 4.7-24.5 mmol/l. In pony I2, the propionic acid concentrations of 27.4-33.1 mmol/l were higher than normal at 6 and 8 h after drug administration, and the

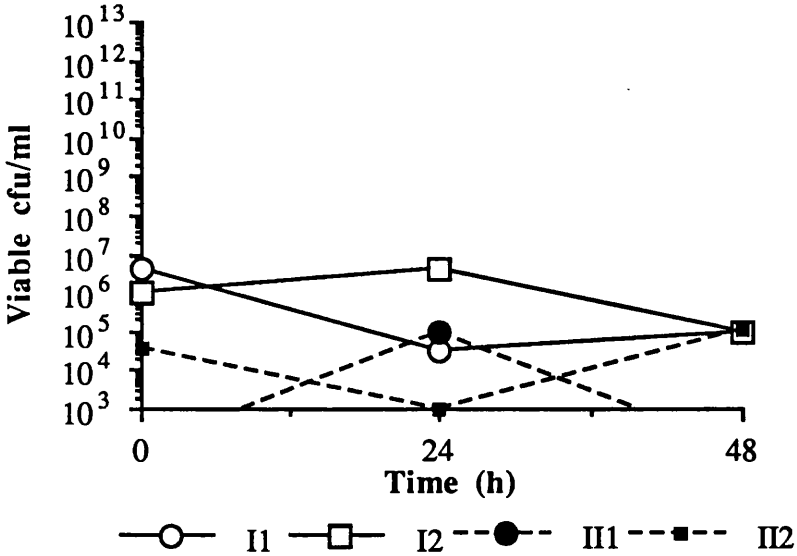

Figure 3-13. Counts of viable *Clostridium spp.* in caecal liquor following intravenous administration of penicillin G to ponies

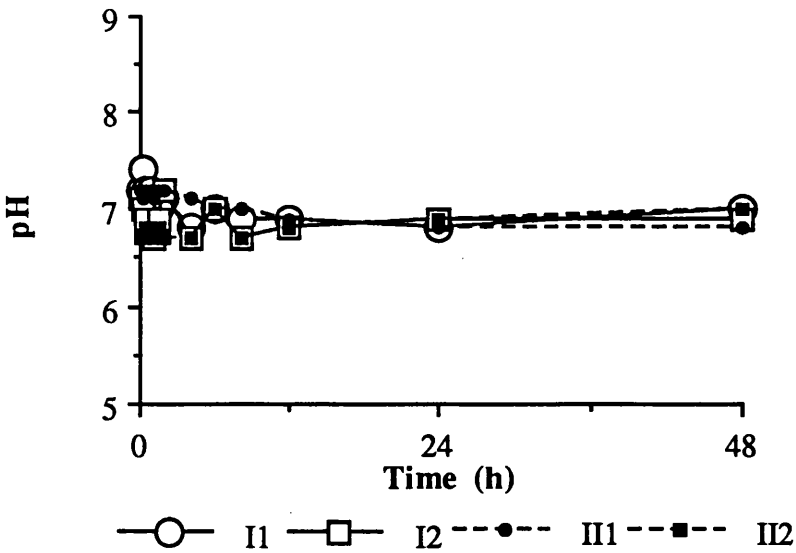

Figure 3-14. Caecal liquor pH following intravenous administration of penicillin G to ponies

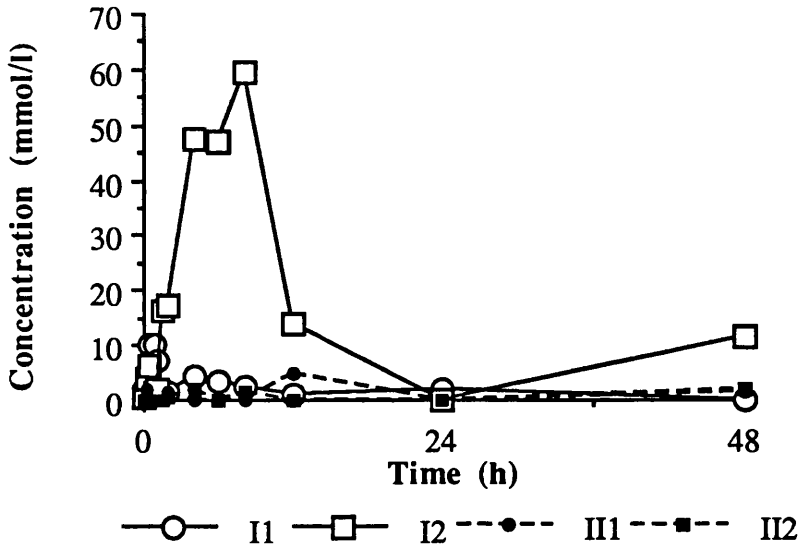

Figure 3-15. Lactic acid concentrations in caecal liquor following intravenous administration of penicillin G to ponies

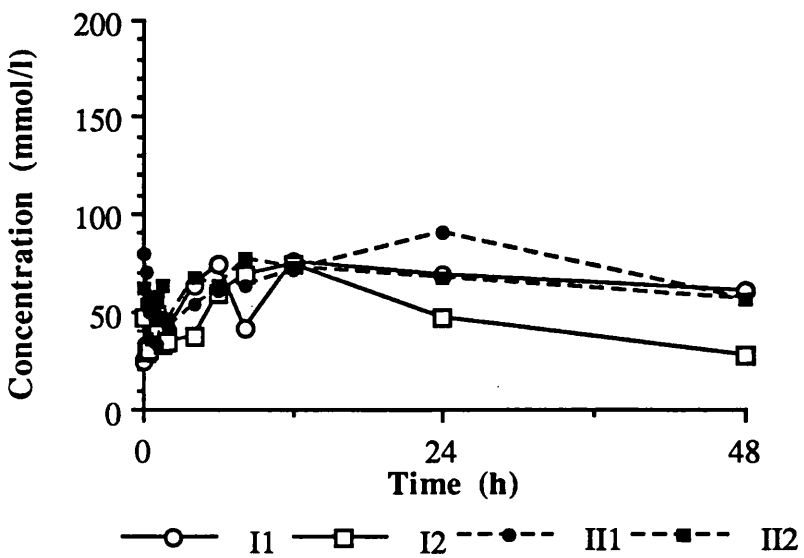

Figure 3-16. Total VFA concentrations in caecal liquor following intravenous administration of penicillin G to ponies

butyric acid concentrations of 0.0-3.0 mmol/l were below the normal range of 4.8-67.3 mmol/l at 0.5, 1.5, 2, 4 and 48 h. In ponies I2 and II2, the isovaleric acid concentrations of 7.5 and 6.3 mmol/l at 0 and 12 h, respectively, were higher than the normal range of 0.0-3.8 mmol/l.

The proportions of acetic, propionic and butyric acids, as a percentage of the total VFA concentrations, are given in Appendix A (Tables A27b, A28b, A29b and A30b). In pony I2, the ratios of acetic and propionic acid were 74.3% at 48 h, and 42.2-58.0% at 1.5, 2, 4, 6, and 8 h, respectively, and were elevated above the normal ranges of 27.9-67.0 % and 4.9-38.5 %, respectively. In pony I2, the ratio of butyric acid of 0.0-5.2% at 2, 4 and 48 h was below the normal range of 8.7-66.7%.

Faecal SCFA concentrations following intravenous administration of penicillin G to ponies are shown in Figure 3-17, and the individual data is given in Appendix A (Tables A31-A34). Lactic acid concentrations were within the normal range of 0.0-24.4 mmol/l at all sample times in all ponies. There was considerable variation in the total and individual VFA concentrations however, there were no trends in VFA concentrations that were considered to be associated with penicillin G administration. The total VFA concentrations were lower than the normal range of 24.4-109.2 mmol/l at 24 and 48 h after drug administration to pony I1, and throughout the study in ponies I2, II1 and II2.

3.4.5 Faecal dry matter content and consistency

Faecal dry matter content versus time following intravenous administration of penicillin G to ponies is shown in Figure 3-18, and the individual data is given in Appendix A (Table A35). There were no marked alterations in faecal dry matter content following intravenous administration of penicillin G. No changes in faecal consistency were observed.

3.4.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix A (Tables A36 and A37). The results of the haematological examinations are given in Appendix A (Tables A38 and A39). There were no alterations in plasma biochemistry or haematological parameters that were considered to be associated with the intravenous administration of penicillin G.

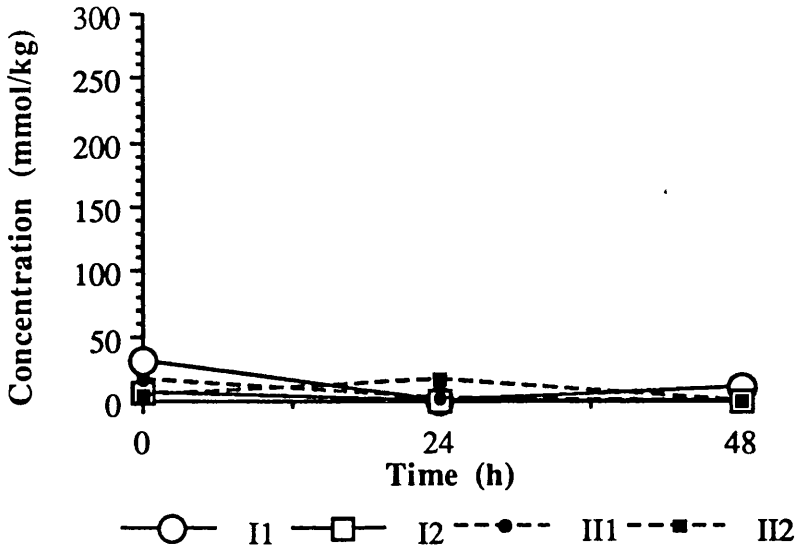

Figure 3-17. Total VFA concentrations in faeces following intravenous administration of penicillin G to ponies

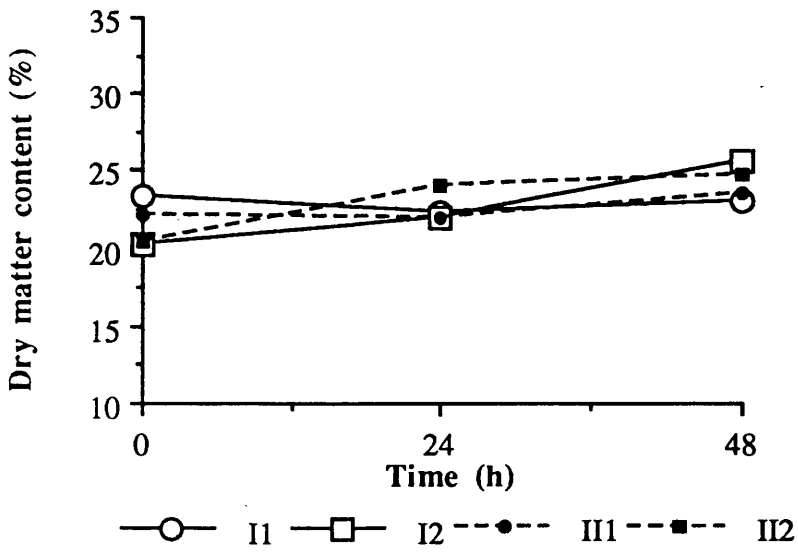

Figure 3-18. Faecal dry matter content following intravenous administration of penicillin G to ponies

3.5 Results of oral administration of penicillin G to ponies with cannulated caecal fistulas

3.5.1 Plasma disposition and pharmacokinetics

The plasma concentrations of penicillin G following oral administration to ponies are shown in Figure 3-19, and the individual data is given Appendix A (Table A40). Maximum plasma concentrations of 0.19, 0.09, 0.14 and 0.16 μg/ml were measured at 0.5 h after nasogastric administration of penicillin G to ponies I1, I2, II1 and II2.

The pharmacokinetic parameters calculated following oral administration of penicillin G to ponies are given in Table 3-3. The plasma concentration versus time data from pony I2 could not be described using a bi-exponential equation. No lag time was required to describe the plasma concentration versus time data following oral administration of penicillin G. The MAT was calculated as the difference between the mean MRT following intravenous administration for each animal and the MRT following oral administration on each occasion. The MAT was quite short in all the animals. The bioavailability of penicillin G, calculated from the AUC_{oral} and the mean $AUC_{intravenous}$, was low (0.12-0.34%) following oral administration.

3.5.2 Caecal liquor and faecal drug concentrations

The caecal liquor concentrations following oral administration of penicillin G to ponies are shown in Figure 3-20, and the individual data is given in Appendix A (Table A41). A maximum caecal liquor concentration of 157.12, 89.73, 118.98 and 4.96 μg/ml was measured at 1.5 h in pony I1, 1 h in pony I2, and 6 h in ponies II1 and II2, respectively. The caecal concentrations were much higher than the plasma concentrations of penicillin G following oral administration.

The disposition of penicillin G in the caecal liquor was described using observed AUC and AUMC to calculate MRT (Table 3-4). The AUC and AUMC were much larger than those calculated for penicillin G in plasma as was the MRT. There was a marked difference in MRT between the 2 animals, a fact that was also reflected in the difference in the time to maximum caecal liquor concentrations.

Faecal penicillin G concentrations following oral administration of penicillin G to ponies are given in Appendix A (Table A42). Faecal drug concentrations were measured at 24 h after oral administration of penicillin G to pony I2 (0.65 μg/g), only.

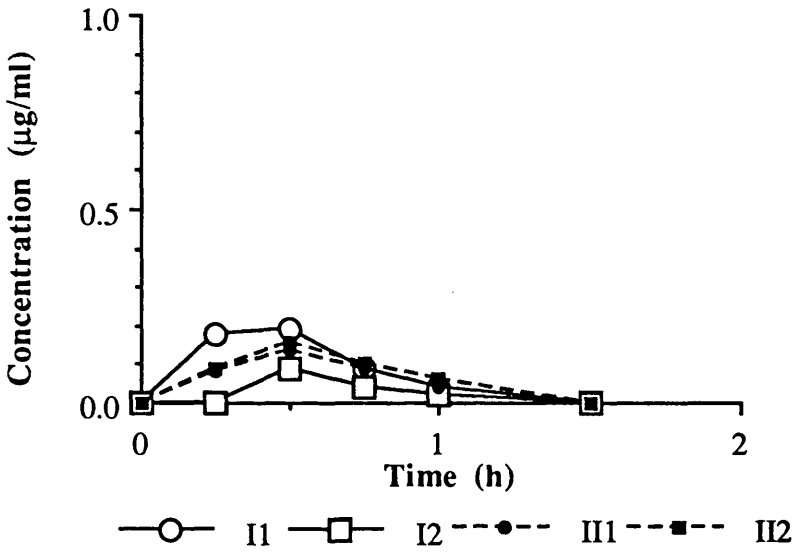

Figure 3-19. Plasma concentrations of penicillin G following oral administration to ponies

Parameter	I1	I2	II1	II2
t1/2 B2 (min)	7.74	-	10.80	11.46
t1/2 B1 (min)	13.32	-	16.62	21.18
C_{max} (µg/ml)	0.19	0.09	0.14	0.16
t_{max} (min)	30	30	30	30
AUC_{obs} (µg.h/ml)	0.13	0.04	0.09	0.11
$AUMC_{obs}$ (µg.h^2/ml)	0.07	0.03	0.05	0.07
AUC (µg.h/ml)	0.12	-	0.07	0.10
AUMC (µg.h^2/ml)	0.06	-	0.05	0.08
MRT (min)	32.31	45.00	33.33	38.18
MAT (min)	-41.36	-28.67	-22.38	-17.53
F (%)	0.27	0.12	0.24	0.34

Table 3-3. Disposition kinetics of penicillin G in plasma following oral administration to ponies

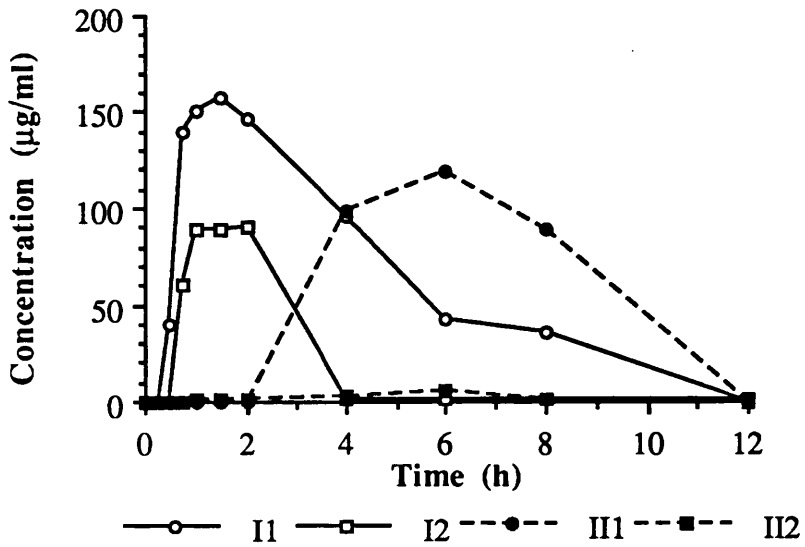

Figure 3-20. Caecal liquor concentrations of penicillin G following oral administration to ponies

Parameter	I1	I2	II1	II2
AUC_{obs} (µg.h/ml)	746.24	230.73	702.73	24.15
$AUMC_{obs}$ (µg.h^2/ml)	2702.93	567.19	4356.80	161.47
MRT (h)	3.62	2.46	6.20	6.69

Table 3-4. Disposition kinetics of penicillin G in caecal liquor following oral administration to ponies

3.5.3 Bacteriological examinations

Salmonella spp. and *C. difficile* were selected for but not isolated.

Counts of viable bacteria in caecal liquor following oral administration of penicillin G are shown in Figures 3-25 to 3-29, and the individual data is given in Appendix A (Table A43).

The viable number of coliforms isolated following oral administration of penicillin G to ponies increased to 10^8-10^{10}/ml (Figure 3-21). This was most marked at 24, 48 and 72 h after drug administration to ponies I1, II1 and II2. There was a slight increase in the viable number of coliforms isolated from pony I2 (up to 10^7/ml) at 24 and 48 h after drug administration.

There was an increase in the number of viable caecal streptococci (up to 10^8-10^{10}/ml) isolated following oral administration of penicillin G (Figure 3-22). There was a slight increase in the number of streptococci isolated (to 10^8/ml) at 24 h after drug administration to ponies I1 and I2, and a marked increase in the number of viable faecal streptococci isolated (10^{10}/ml) at 48 h after drug administration to pony II1. There was a very small increase in the number of streptococci isolated (10^7/ml) at 48 h after drug administration to pony II2.

There were no marked alterations in the number of viable lactobacilli isolated from caecal liquor, although there was an increase in the number of lactobacilli isolated (to 10^9/ml) at 48 h after oral administration of penicillin G to pony II1 (Figure 3-23). In ponies I2 and II1, there were no lactobacilli counted at 72 h after drug administration due to mould overgrowth on the microaerophilic plates. Lactobacilli identified using the API system were *L. acidophilus*, *L. fermentum* and *L. jensenii*.

There were no alterations in the number of viable *Bacteroides spp.* isolated from ponies I1, II1 and II2 after drug administration (Figure 3-24). *Bacteroides spp.* were isolated at 48 h after drug administration to pony I2, but they could not be counted due to the drops of diluted caecal liquor having run together on the agar plate. *Bacteroides spp.* counts were reduced slightly (to 10^8/ml) at 72 and 168 h after drug administration to pony I2. *Bacteroides spp.* identified using the API system were *B. eggerthii*, *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*.

There was an increase (10^6-10^8/ml) in the number of *Clostridium spp.* isolated following oral administration of penicillin G to ponies (Figure 3-25). There were high numbers of *Clostridium spp.* isolated (10^6-10^8/ml) from caecal liquor at 24, 48 and 168 h in pony I1, at 0, 24, 48, 72 and 96 h in pony I2, at 24, 48 and 72 h in pony II1, and at 48, 72 and 96 h in

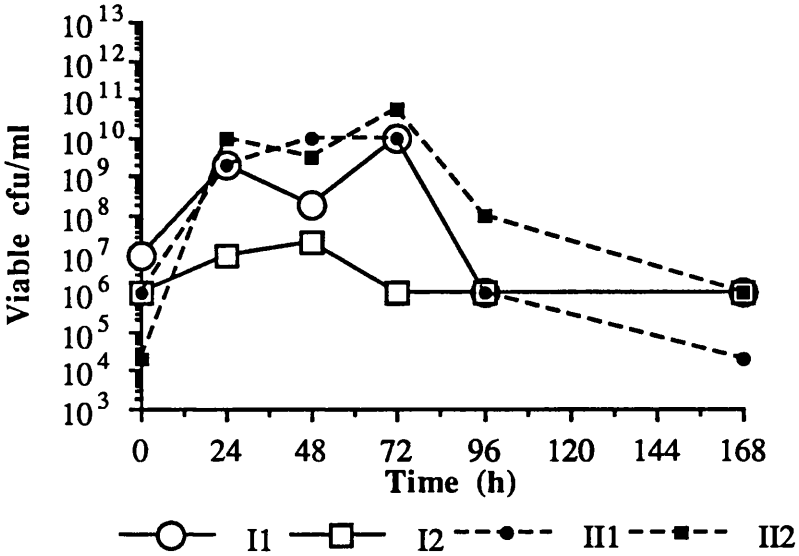

Figure 3-21. Counts of viable coliforms in caecal liquor following oral administration of penicillin G to ponies

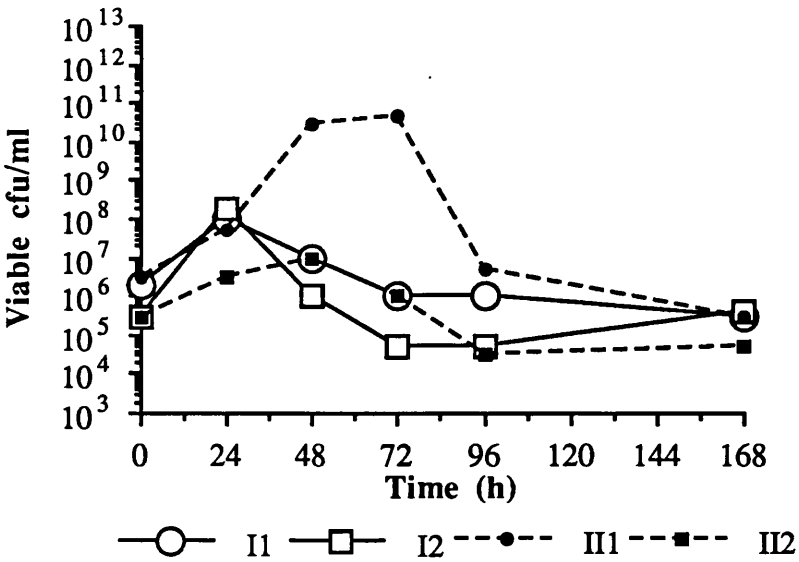

Figure 3-22. Counts of viable streptococci in caecal liquor following oral administration of penicillin G to ponies

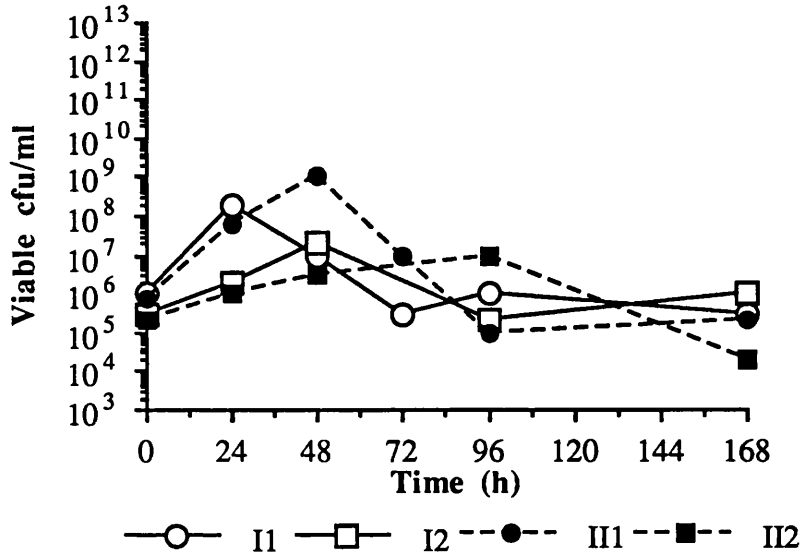

Figure 3-23. Counts of viable lactobacilli in caecal liquor following oral administration of penicillin G to ponies

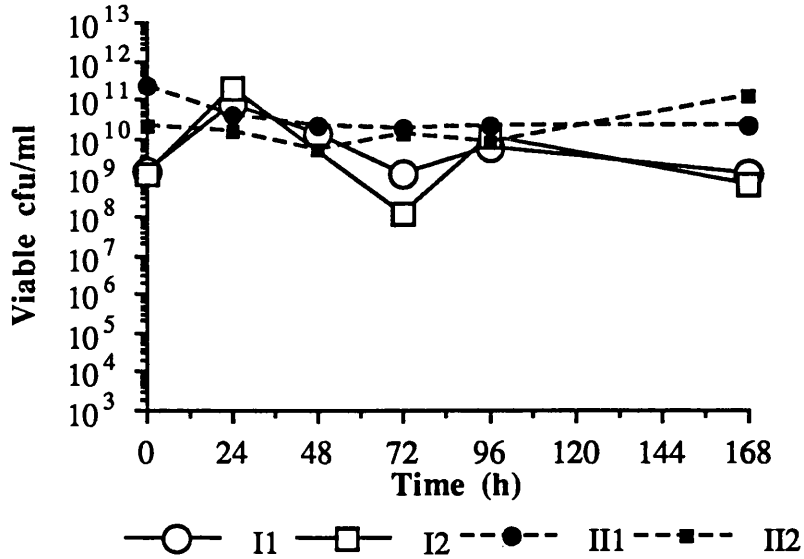

Figure 3-24. Counts of viable *Bacteroides spp.* in caecal liquor following oral administration of penicillin G to ponies

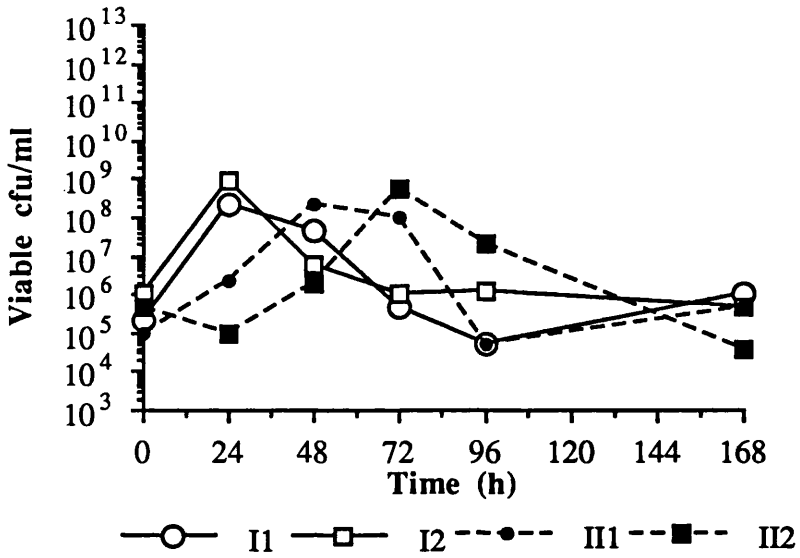

Figure 3-25. Counts of viable *Clostridium spp.* in caecal liquor following oral administration of penicillin G to ponies

pony II2. *Clostridium spp.* identified using the API system were *C. butyricum*, *C. clostridiiforme*, and *C. perfringens*. The latter was identified from pony III (10^6/ml) at 24 h after oral administration of penicillin G.

Other bacteria identified using the API system were *Actinomyces israelii*, *Bifidobacter adolescentis*, *Eubacterium lentum*, and *Peptococcus spp.*.

3.5.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

The luminal pH measurements are shown in Figure 3-26, and the individual data is given Appendix A (Table A44). In pony I1 the caecal liquor pH was increased (>7.2), up to a maximum of pH 8.3, at 2, 4, 6, 8, 12 and 24 h after drug administration. In pony I2, the caecal liquor pH was increased (>7.2), up to a maximum of pH 7.6, at 0.75, 1, 1.5, 4, 6, and 72 h after drug administration. There was an increase in the caecal liquor pH in pony II1 at 4 and 6 h after drug administration up to 7.3 and 7.5, respectively. There was a slight increase in the caecal liquor pH (7.3) at 6 h after drug administration to pony II2.

Caecal liquor SCFA concentrations are shown in Figures 3-27 and 3-28, and the individual data is given in Appendix A (Tables A45a-A48a).

In pony I1, caecal liquor lactic acid concentrations were increased to 10.1-38.7 mmol/l at 4, 6, 8, 12, 24, 28 and 32 h, and were above the normal range of 0.0-24.4 mmol/l at 8 h after drug administration (Figure 3-27). In ponies I2, II1 and II2, caecal liquor lactic acid concentrations were elevated to 5.1-11.1 mmol/l at 4, 8, 12 and 24 h, at 12, 24, 28 and 48 h, and at 12, 24, 28 and 32 h, respectively, after drug administration but remained within the established normal range.

The total and individual VFA concentrations fluctuated around the normal ranges (Figure 3-28). In ponies I1 and I2, total VFA concentrations of 17.2-19.9 mmol/l at 24 h and at 12, 28 and 72 h, respectively, were lower than the normal range of 24.4-109.2 mmol/l. There was a reduction in acetic acid concentrations, below the normal range of 12.6-64.5 mmol/l, to 4.3-10.9 mmol/l at 8, 12 and 24 h in pony I1 and at 4, 6 and 8 h in pony I2. Propionic acid concentrations, compared with the normal range of 4.7-24.5 mmol/l, increased to 36.4-60.7 mmol/l at 4, 6 and 8 h in ponies I1 and I2. The concentration of propionic acid fell to 0.0-3.7 mmol/l at 24 h in pony I1, at 0.25, 0.5, 0.75, 1, 1.5, 12, 24, 28 and 72 h in pony I2, at 24 h in pony II1, and at 12 h in pony II2. Butyric acid concentrations fell, below the normal range of 4.8-67.3 mmol/l, to 0.0-3.5 mmol/l at 6, 8, 12 and 168 h in pony I1, at , 6, 8, 12, 28 and 72 h in pony I2, at 8, 12, and 32 h in pony II1, and at 28 h in pony II2.

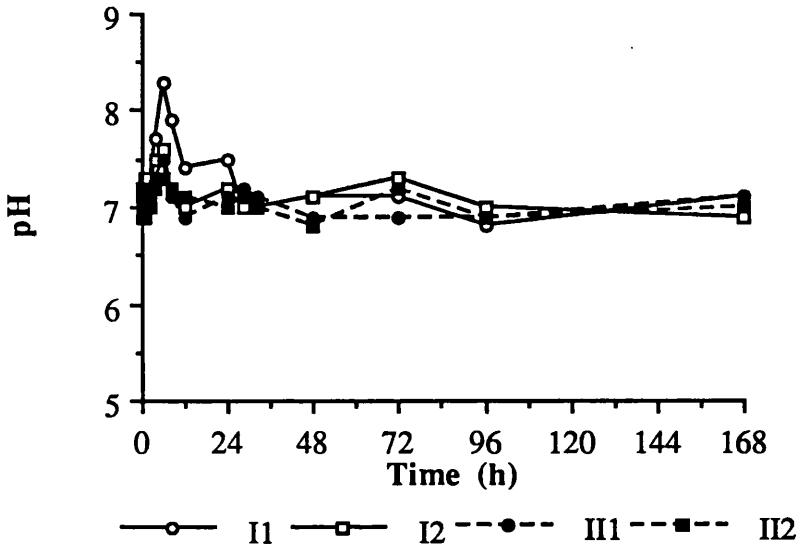

Figure 3-26. Caecal liquor pH following oral administration of penicillin G to ponies

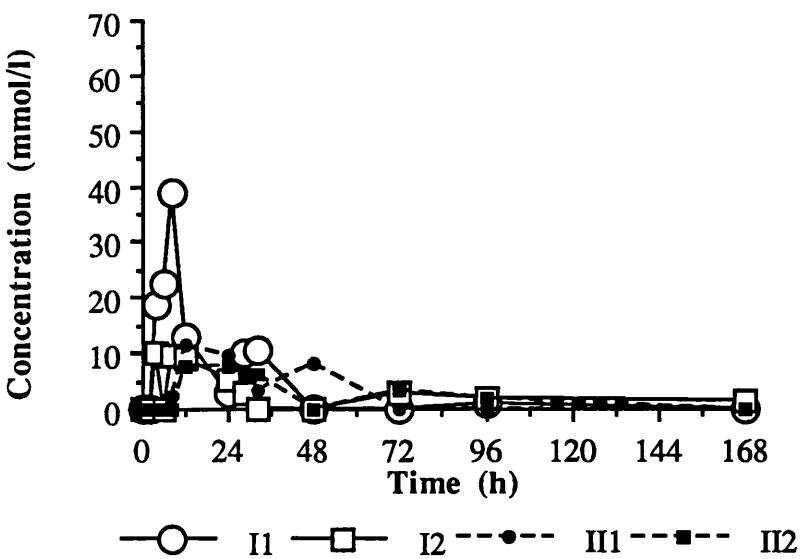

Figure 3-27. Lactic acid concentrations in caecal liquor following oral administration of penicillin G to ponies

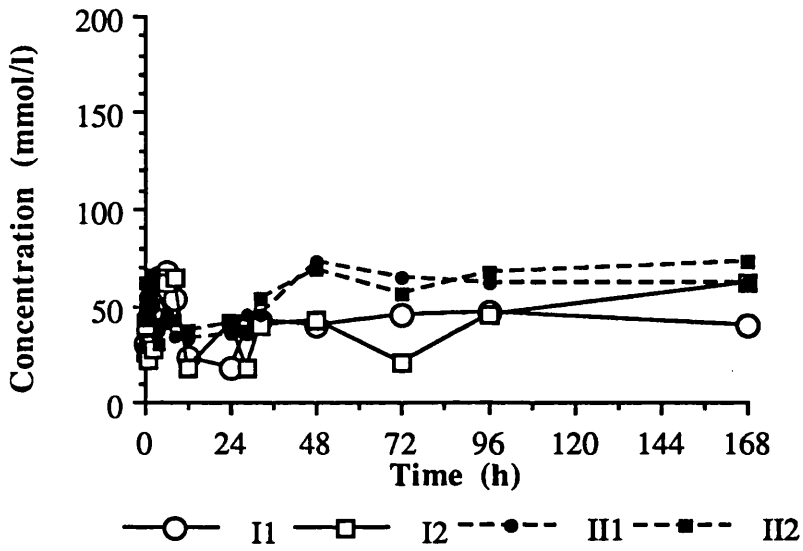

Figure 3-28. Total VFA concentrations in caecal liquor following oral administration of penicillin G to ponies

The proportions of acetic, propionic and butyric acids, as a percentage of the total VFA concentrations are given in Appendix A (Tables A45b, A46b, A47b and A48b). There was a reduction in the ratio of acetic acid to 6.6-16.1%, compared with the normal range of 27.9-67.0%, at 6 and 8 h in pony I1 and at 4, 6 and 8 h in pony I2. The ratio of acetic acid increased to 71.1-86.7% at 28 h in pony I2, at 8, 12 and 24 h in pony II1, and at 8, 12 and 28 h in pony II2. The ratio of propionic acid was increased to 56.0-93.4%, compared with the normal range of 4.9-38.5%, at 4, 6, 8 and 12 h in pony I1 and at 4, 6 and 8 h in pony I2. There was no propionic acid at 12 h in ponies I2 and II2 and at 24 h in pony II1. There was a reduction in the ratio of butyric acid to 0.0-7.1%, compared with the normal range of 8.7-66.7%, at 6, 8 and 12 h in pony I1, at 6 and 8 h in pony I2, at 8, 12 and 32 h in pony II1, and at 28 and 72 h in pony II2. There was an increase in the percentage of propionic plus butyric acid to 77.7-93.4%, compared with the normal range of 27.1-72.1%, at 4, 6 and 8 h in pony I1 and at 4, 6 and 8 h in pony I2. The ratio of propionic plus butyric acid fell to 13.3-19.9%, compared with the normal range of 27.1-72.1%, at 12 h in pony I2, at 8, 24, 28 and 32 h in pony II1, and at 8 h in pony II1.

Faecal SCFA concentrations are shown in Figures 3-29 and 3-30, and the individual data is given in Appendix A (Tables A49-A52). Faecal lactic acid concentrations were increased to 6.2-6.5 mmol/kg at 0 and 72 h after drug administration to pony I1, but there were no lactic acid concentrations outside the normal range of 0.0-24.4 mmol/l following oral administration of penicillin G to ponies I1, I2, II1 and II2 (Figure 3.29). There were considerable variations in the faecal total and individual VFA concentrations following oral administration of penicillin G (Figure 3.30). In pony I1, the faecal total VFA concentrations were lower than the normal range of 24.4-109.2 mmol/l throughout the study. In pony I2, the total VFA concentrations were lower than normal at 0, 24, 72, 96 and 168 h after drug administration. In pony II1, the faecal total VFA concentrations were lower than normal at 0, 24 and 168 h after oral administration of penicillin G. In pony II2, the faecal total VFA concentrations were lower than normal at all sampling times except 168 h.

3.5.5 Faecal dry matter content and consistency

The faecal dry matter content following oral administration of penicillin G is shown in Figure 3-31, and the individual data is given in Appendix A (Table A53). There were slight changes in faecal dry matter content, and no alterations in faecal consistency were observed.

3.5.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix A (Tables A54-A57). There was an increase in plasma urea concentrations up to 7.1 mmol/l following oral administration of

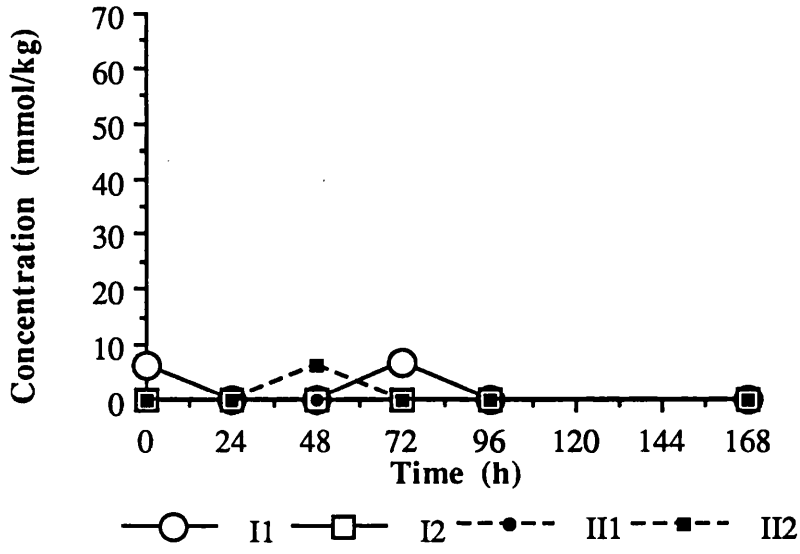

Figure 3-29. Lactic acid concentrations in faeces following oral administration of penicillin G to ponies

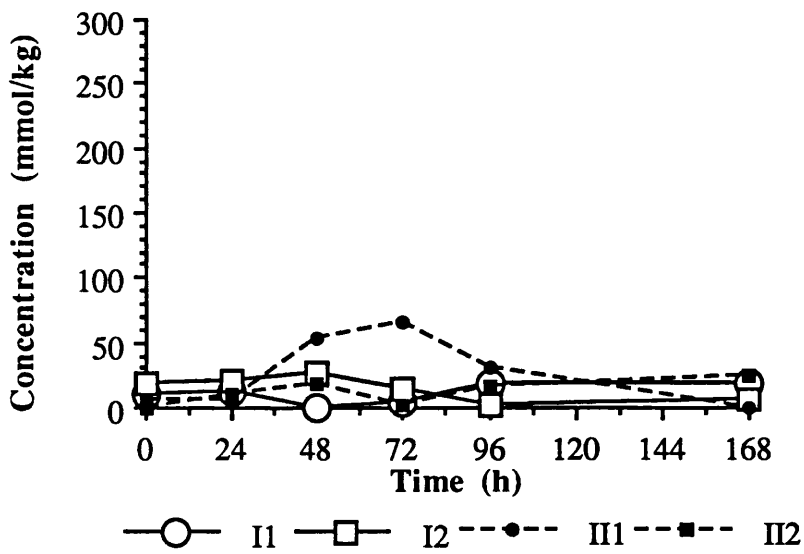

Figure 3-30. Total VFA concentrations in faeces following oral administration of penicillin G to ponies

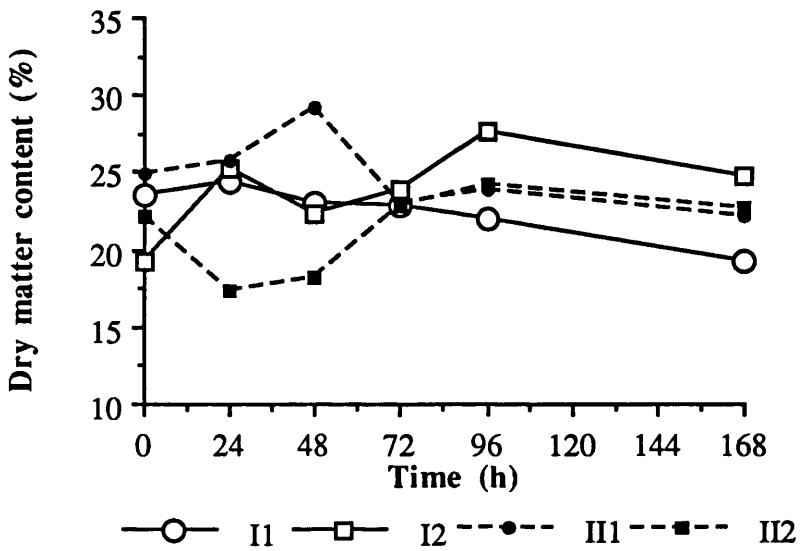

Figure 3-31. Faecal dry matter content following oral administration of penicillin G to ponies

penicillin G to pony I on occasions 1 and 2. The plasma bilirubin concentration was elevated to 30 μmol/l at 24 h in pony II1. The results of the haematological examinations are given in Appendix A (Tables A58-A61). Other variations in plasma biochemistry and in the haematological parameters were not considered to be associated with the administration of penicillin G.

3.6 Results of *in vitro* studies with penicillin G

3.6.1 Caecal liquor concentrations

Penicillin G concentrations (mean±SEM) in caecal liquor following incubation *in vitro* are shown in Figure 3-32, and the individual and mean data are given in Appendix A (Tables A62a and b). After 3 h incubation, there was an average of 80.2 % of drug remaining. However, the largest reductions in concentration (zone diameter) were seen at either end of the concentration range due to limitations of the assay technique (zone diameter, limit of detection). That is, around 50-60% of penicillin G remaining at initial concentrations of 0.25 and 1 μg/ml, whilst around 100% the drug activity remained at 5, 10 and 20 μg/ml, and 70-80% of the drug activity remained at 40 and 80 μg/ml. There was an average of 35.5% (excluding 0.25 and 1 μg/ml samples) of drug remaining following 24 h incubation. Following 24 h incubation, there was a similar percentage reduction in concentration in all the samples (25-50%), except there was no inhibition zone for the 0.25 μg/ml sample, and there was only 1% of the 1 μg/ml sample remaining.

3.6.2 SCFA concentrations

Caecal lactic acid and total VFA concentrations (mean±SEM) following *in vitro* incubation are shown in Figure 3-33, and the individual and mean data are given in Appendix A (Tables A63-A67). There were no alterations in mean lactic acid or mean total VFA concentrations with time or drug concentration.

3.6.3 Acid pH

A plot of penicillin G concentrations (mean±SEM) remaining following *in vitro* incubation for 1 h at pH 1.9 versus initial penicillin G concentrations is shown in Figure 3-34, and the individual and mean data are given in Appendix A (Table A68). There was a large amount of penicillin G destroyed by incubation at acid pH; on average there was only 15.3% of drug remaining. There may be some variation in the percentage of drug activity destroyed at different initial concentrations; 9, 12, 16 and 24% of drug remained (mean) at 1, 2, 5 and 10 μg/ml respectively. However, this may be associated with the assay technique.

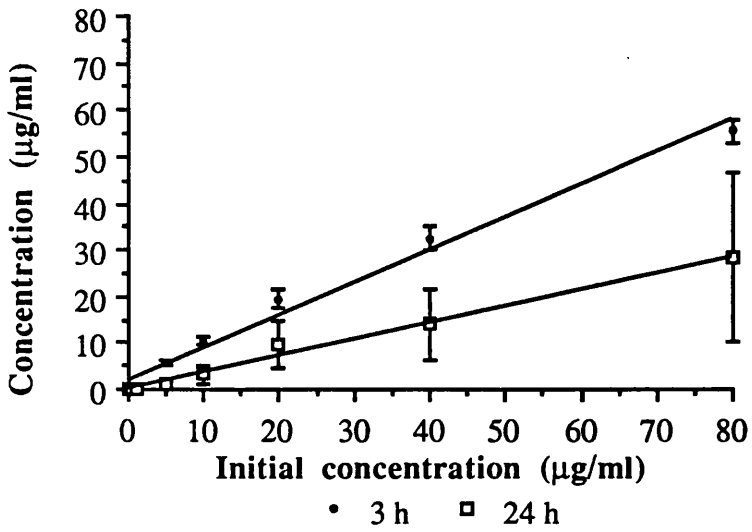

Figure 3-32. Concentrations (mean±SEM) of penicillin G in caecal liquor following incubation *in vitro* for 3 and 24 h

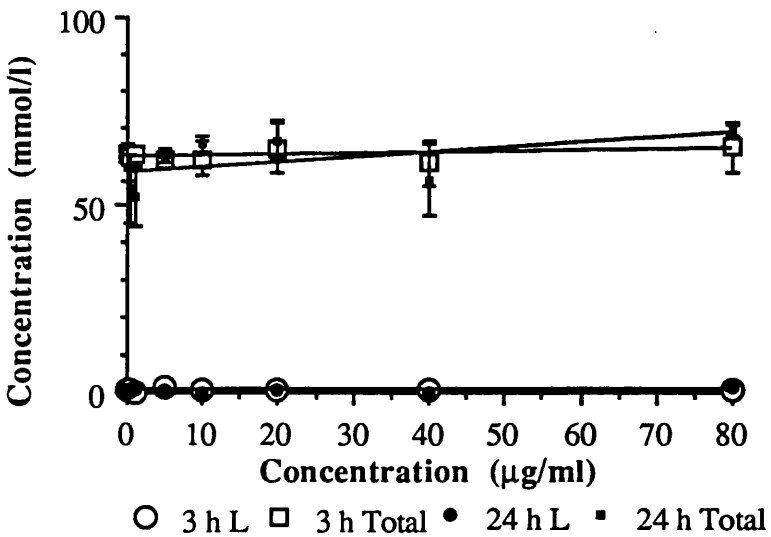

Figure 3-33. SCFA concentrations (mean±SEM) in caecal liquor following incubation *in vitro* with penicillin G for 3 and 24 h

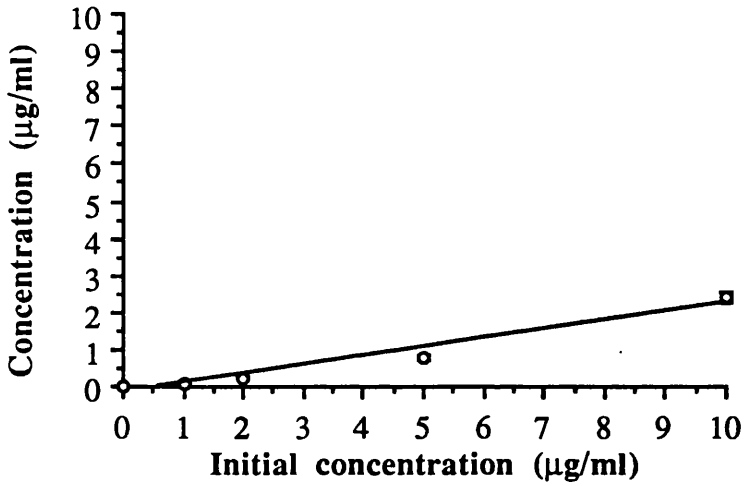

Figure 3-34. Concentrations (mean±SEM) of penicillin G following incubation at pH 1.9 for 1 h

3.6.4 Binding to hay

A plot of penicillin G concentrations remaining following *in vitro* incubation with chopped hay at pH 1.9 and pH 7.0 is shown in Figure 3-35. There was only 1 replicate of this study performed. There was a range of concentrations remaining following incubation at pH 1.9, namely 1.05 1.06, 1.43 and 0.89 µg/ml of the initial 10, 25, 50 and 100 µg/ml. There was a wide range of concentrations remaining following incubation at pH 7.0, namely 0.21, 0.25, 7.65 and 9.84 µg/ml of the initial 10, 25, 50 and 100 µg/ml. However, it is interesting that only 10.49, 10.57, 14.34 and 8.85% or an average of 11.06% remained at pH 1.9, and 2.1, 1, 15.3 and 9.84% or an average of 7.1 % remained at pH 7.0.

3.7 Discussion

Dürr (1976) calculated the elimination half-life of penicillin G to be 53.3±4.5 min (mean ±SEM) following intravenous administration of penicillin G at dose rates of 21 and 36.3 mg/kg bwt. Whereas, Knight (1975) noted that the elimination half-life of potassium penicillin G was 47 min following intravenous administration at dose rates of 12.5, 18 or 37.6 mg/kg bwt. In the present study, the elimination half-life (harmonic mean) of penicillin G was 38.95, 27.25 and 31.52 min in horses, ponies and donkeys, respectively. However, the elimination half-life of penicillin G following intravenous administration to ponies I1, I2, II1 and II2 was 69.60, 38.82, 36.78 and 62.70 min respectively. The long elimination half-life calculated for ponies I1 and II2 may reflect perivenous administration but it was not much longer than the elimination half-life calculated following intravenous administration of penicillin G to horses by Dürr (1976). Although the elimination half-life of penicillin G reported in the present study following intravenous administration to horses, ponies and donkeys was shorter than the values reported by other authors, it may not affect the regime required to maintain adequate therapeutic plasma concentrations of penicillin G.

Knight (1975) stated that a plasma or serum concentration of drug 2-4 times the MIC was required to ensure the bactericidal activity of a penicillin. The MIC of susceptible equine Gram positive and Gram negative aerobic bacteria is ≤1 µg/ml penicillin G (Adamson *et al.*, 1985). Similarly, Knight (1975) stated that most bacteria susceptible to penicillin G had an MIC of 0.05-1 µg/ml and that these included ß haemolytic streptococci, non-ß-lactamase producing staphylococci and *C. tetani*. Other organisms, such as *Rhodococcus equi* (MIC ≤4 µg/ml) (Prescott , 1991, Prescott and Nicholson, 1984) are less susceptible to penicillin G. If plasma concentrations are to be maintained above a minimum concentration, for susceptible bacteria, of 0.1 µg/ml (twice 0.05 µg/ml) then intravenous administration of penicillin G at a dose rate of 10 mg/kg bwt should be repeated every 4 h in horses, ponies and donkeys.

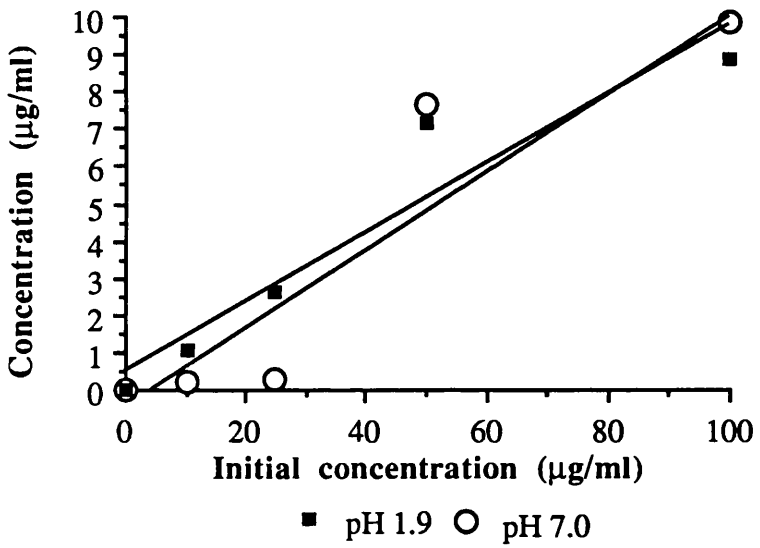

Figure 3-35. Concentrations of penicillin G following incubation *in vitro* with hay at pH 1.9 and pH 7.0 for 3 h

Generally, the computation of optimum drug dose rates tries to maintain plasma or serum drug concentrations at several times above the MIC of an organism and ignores the post-antibiotic effect (Prescott and Baggot, 1985). The so-called post-antibiotic effect is defined as the absence of bacterial growth for several hours after the removal of a drug which was present at or above the MIC. The penicillins act by binding covalently and irreversibly to the active site of the cross-linking enzyme in bacterial cell walls and therefore affect bacteria that are growing rapidly. However, the effect of the penicillins continues even when bacteria are no longer in the active growth phase since drug remains bound to the receptor sites (Tobin, 1979). The post-antibiotic effect is more marked *in vivo* than *in vitro* (Prescott and Baggot, 1985). If plasma concentrations are allowed to fall temporarily below the MIC (0.05 µg/ml) then intravenous administration of penicillin G at a dose rate of 10 mg/kg bwt would need to be repeated less frequently than 6 times per day. Thus, intravenous administration of penicillin G every 6 or 8 h would probably be adequate. This is similar to the inter-dosing interval of 6 h suggested by Knight (1975), although the dose rates of 30-250 mg/kg bwt were much higher than the dose rate used in the present study. Following intravenous administration of penicillin G at a dose rate of 10 mg/kg bwt the Cp0 (mean±SEM) values were 62.90±8.87, 74.52±14.65 and 126.42±43.13 µg/ml in horses, ponies and donkeys, respectively. The Cp0 following intravenous administration of penicillin G at a dose rate of 30-250 mg/kg bwt would be very high. This may be of no additional benefit since there is an optimum concentration above which there is no increase in the rate of bacterial killing (Prescott and Baggot, 1985).

The amount of the total dose of penicillin G in caecal liquor following intravenous administration can be expressed as a percentage of the AUC_{obs} in plasma taking into account the differences between the estimated volume of caecal contents (6.67 and 6.17 l in ponies I and II, respectively) and the estimated plasma volume of 51 ml/kg bwt (Swenson, 1977) which was equal to 11.88 and 11.02 l, in ponies I and II, respectively. Penicillin G (1.95%) was detected in the caecal liquor of 1 animal only following intravenous administration. This suggests that penicillin G was excreted in the bile into the intestinal tract, although entry into the intestinal lumen by other routes is not ruled out. In the other ponies it is possible that penicillin G was present within the intestinal lumen following intravenous administration and was destroyed prior to the analysis of samples. Other authors have shown that penicillin G is inactivated rapidly in the gastrointestinal tract (Prins, 1987) and by ßlactamase producing organisms (De Louvois and Hurley, 1977). ßeta-lactamase production has been described for a number of different organisms, including coliforms and *Bacteroides spp.* which were present in high numbers in the caecal liquor of ponies in the present study (De Louvois and Hurley, 1977).

Penicillin G was absorbed rapidly (maximum plasma concentrations at 0.5 h after administration) following oral administration to ponies. This is similar to the results of the study by Schwark *et al.* (1983) who reported maximum plasma concentrations at 0.5-1 h after oral administration of penicillin V. The bioavailability of penicillin G following oral administration to ponies was low (0.12-0.34%). This was probably due to destruction of the drug by gastric acid since only 15% of penicillin G remained following *in vitro* incubation at pH 1.9 for 1 h. In addition, penicillin G appears to be bound to hay. Watson (1986) reviewed the influence of food on the absorption of antimicrobial agents and noted that, in man, the systemic availability of both penicillin G and penicillin V were reduced by the presence of food in the intestine. In the present study, drug administration took place prior to the ponies receiving their morning hay. Schwark *et al.* (1983) withheld food from horses for 8 h prior to drug administration. The results may have been different if food had been withheld prior to drug administration, however it is unlikely that this would be practical in a clinical situation.

Some penicillin G was absorbed from the gastrointestinal tract following oral administration but the maximum plasma concentrations were low; 0.19, 0.09, 0.14 and 0.16 μg/ml in ponies I1, I2, II1 and II2, respectively. Although these plasma concentrations would be sufficient to treat infections caused by susceptible bacteria, the short MRT (32.31, 45.00, 33.33 and 38.18 min in ponies I1, I2, II1 and II2, respectively) would require very frequent repetition of drug administration, at a dose rate of 10 mg/kg bwt, to maintain adequate therapeutic concentrations in plasma. Schwark *et al.* (1983) and Ducharme *et al.* (1983) administered a much higher dose rate of penicillin V, than the dose rate of penicillin G used in the present study, to obtain plasma concentrations that would be useful clinically. However, Baggot *et al.* (1990) reported that not only was there poor systemic availability of penicillin G and penicillin V, following oral administration at a dose rate of 90 mg/kg bwt to horses, but also that the animals developed clinical signs culminating in the development of profuse watery diarrhoea at 8 h after drug administration. Thus, an increase in the dose rate may not only fail to produce adequate plasma concentrations for therapy, but also increase the likelihood of the development of antimicrobial-associated diarrhoea.

There were high concentrations of penicillin G measured in caecal liquor following oral administration. This was surprising since 85% of penicillin G appeared to be destroyed at a pH similar to that found in the stomach of the equine (pH 1.63-1.97) (Sangiah *et al.*, 1989). However, other authors have reported that gastric pH is much higher than this when digesta are present (Bogan *et al.*, 1984). In the present study it was shown that penicillin G was destroyed or inactivated by caecal liquor *in vitro*. *In vivo*, this appeared to occur following oral administration of drug to one animal only. However, alterations in the number of viable

bacteria isolated from caecal liquor and in the concentrations of SCFAs were similar in both ponies on both occasions.

The time to maximum caecal liquor concentrations of penicillin G varied quite markedly between animals. The peak concentration occurred at 1.5 and 1 h in ponies I1 and I2, and at 6 h after oral administration to ponies II1 and II2. This suggests that gastric and small intestinal transit times may differ markedly in individual animals. There was a wide variation (24.15-746.24 μg.h/ml) in the amount of penicillin (AUC) in the caecal liquor and in the length of time that penicillin remained (MRT) in the caecum (2.46-6.69 h) following oral administration. Although, the MRT was similar between occasions in the two ponies despite the differences in AUC.

The coliform counts recorded in the present study fall into both the range of 10^3-10^4/g faeces described by Smith (1965) and around 10^6/g faeces described by White and Prior (1982) but were much lower than the control values used by Garner *et al.* (1978). There were no consistent alterations in the number of coliforms isolated following intravenous administration of penicillin G to horses, ponies and donkeys or ponies with cannulated caecal fistulas. There were apparent increases in the mean number of coliforms isolated from pony and donkey faeces following intravenous administration of penicillin G. In addition, high caecal liquor coliform counts of up to 10^9/ml were recorded for up to 168 h after oral administration of penicillin G to ponies with cannulated caecal fistulas. In previous studies, Andersson *et al.* (1971) recorded an increase in the number of coliforms isolated from faeces 1-2 days after intravenous administration of oxytetracycline at a very high dose rate to horses, and White and Prior (1982) recorded viable coliform counts of around 10^9/g faeces following repeated oral administration of high doses of oxytetracycline. In addition, Andersson *et al.* (1971) and White and Prior (1982) found significant alterations in the number of faecal streptococci isolated following oxytetracycline administration. There were no marked alterations in the number of viable streptococci isolated following the intravenous administration of penicillin G, but there were increases in the number of viable streptococci isolated following oral administration of penicillin G, in the present study. There were increases in the number of *Clostridium spp.* isolated following intravenous administration of penicillin G to donkeys and following oral administration of penicillin G to ponies. It is interesting that there were increases in the number of bacteria that are susceptible to penicillin G *in vitro*, such as streptococci and *Clostridium spp.*.

Andersson *et al.* (1971) reported an increase in faecal pH from 6.1-6.5 to 6.9-8.1 following intravenous administration of a massive dose of oxytetracycline to horses. The control caecal pH range noted by Garner *et al.* (1978) was around 7.2. In the present study there was a

modest increase in caecal liquor pH following oral administration of penicillin G with a maximum pH of 8.3, 7.6, 7.5 and 7.3 in ponies I1, I2, II1 and II2 respectively.

There were alterations in SCFA concentrations following both intravenous and oral administration of penicillin G. There were increases in lactic acid concentrations following both intravenous and oral administration of penicillin G, although these changes appeared mainly in the caecal liquor and not in the faecal samples, suggesting that the excess lactic acid was absorbed across the gastrointestinal mucosa. The increase in lactic acid concentrations could be explained by the increase in the number of lactic acid producing bacteria, such as streptococci and *Clostridium spp.*. The increased lactic acid concentrations occurred at variable times after intravenous or oral administration of penicillin G, although they occurred after the peak drug concentrations. It is interesting that the highest concentration of lactic acid was measured following intravenous administration of penicillin G to pony I2. This was the only animal in which penicillin G was measured in caecal liquor following intravenous administration. There was only one pony in which lactic acid concentrations were higher than the normal range of 0.0-24.4 mmol/l following oral administration of penicillin G, and there was a slight increase in the lactic acid concentration in the faeces of this pony.

There were no consistent alterations in VFA concentrations or proportions following intravenous administration of penicillin G. However, in pony I2, the only pony in which penicillin G was measured in the caecal liquor following intravenous administration, propionic concentrations increased, butyric acid concentrations decreased, the proportions of acetic and propionic acids increased and the proportion of butyric acid decreased. There was a reduction in the concentrations of propionic and butyric acids, and a reduction in the proportion of butyric acid in caecal liquor, following oral administration of penicillin G. In addition, there was a reduction in the proportions of propionic plus butyric acids, which reflected a relative increase in the amount of acetic acid and a relative reduction in propionic and butyric acid concentrations. Prins (1987) noted that although penicillin G was inactivated rapidly by rumen contents, administration of the drug still produced a substantial reduction in the cellulolytic activity of rumen fluid. Interestingly, the high pH and low propionic and butyric acid concentrations occurred at similar times (between 6 and 24 h after drug administration). Although, there was a reduction in the total VFA concentrations in only two ponies, the alterations in the proportions of VFA concentrations may represent a reduction in production of VFA and hence explain the apparent increase in pH.

Alterations in propionic and butyric acid concentrations are associated with the administration of the so-called growth promoting antimicrobial agents. Generally, there is a shift in fermentation towards propionic acid production with reductions in methanogenesis

and acetic and butyric acid concentrations. However, a reduction in propionic acid concentrations has been reported following administration of chloral hydrate to ruminants (Amgarten *et al.*, 1981). The reduction in propionic acid and increase in lactic acid concentrations were attributed to inhibition of the reductive pathway from lactate to propionate. Propionic acid is produced, along with acetate and succinate, by *Bacteroides spp.* and *Selenomonas spp.* (Miller and Wolin, 1979). Butyric acid is formed by a variety of different bacteria in the gastrointestinal tract, including *C. butyricum*, *Eubacterium spp.*, *Butyrivibrio spp.*, *Fusobacterium spp.* and *Bacteroides spp.* (Miller and Wolin, 1979). It may be that the increase in the number of coliforms, streptococci and *Clostridium spp.* resulted in a relative reduction in the numbers of propionic and butyric acid producing bacteria.

An increase in intestinal lactic acid concentrations may be important in the development of systemic lactic acidosis and its clinical sequelae. It has been shown that butyric acid is an important respiratory fuel in colonocytes (Roediger, 1982) and a reduction in colonic butyric acid concentrations has been associated with adenomatous polyps and colonic cancer in man (Clausen *et al.*, 1991). A prolonged reduction in butyric acid concentrations may result in a reduction in the energy supply to intestinal cells and the development of large intestinal disease. In addition, a prolonged elevation of intestinal pH removes the antibacterial effect of acetic and formic acids (pH 6.8 or less) and may promote bacterial overgrowth (Fleming and Arce, 1986).

There was an increase in the plasma urea concentrations following oral administration of penicillin G to pony I in the present study. In addition, there was an increase in the plasma bilirubin concentration at 24 h following oral administration of penicillin G to pony II1. These alterations in clinical chemistry were noted by Andersson *et al.* (1971) following intravenous administration of a massive dose of oxytetracycline, although none of the other changes noted previously were seen in the present study. Moreover, there were no clinical signs associated with the onset of antimicrobial-associated diarrhoea and no alterations in either faecal dry matter content or consistency were observed.

The intravenous administration of penicillin G at a dose rate of 10 mg/kg bwt repeated every 6-8 h would be suitable to treat infections due to susceptible bacteria. There is a low risk of developing antimicrobial-associated alterations in gastrointestinal microbial metabolism following a single intravenous administration of penicillin G at a dose rate of 10 mg/kg bwt. Oral administration of penicillin G at a dose rate of 10 mg/kg bwt would be unsuitable for systemic antimicrobial therapy of susceptible infections in the equine due to low systemic availability and to the alterations in gastrointestinal microbial metabolism caused by unabsorbed active drug remaining in the gastrointestinal lumen.

4 Studies with ampicillin

4.1 Introduction

A number of penicillins, including ampicillin and amoxicillin, have an extended spectrum of activity, in that they retain the typical activity of penicillin G against Gram positive bacteria and, in addition, are active against some Gram negative bacteria not affected by penicillin G, but they are not active against ß-lactamase producing bacteria (Brander, 1977, Brumbaugh, 1987).

Ampicillin has been used in the treatment of many conditions in the equine, including enteritis, metritis, pneumonia and septicaemia (Arbeiter *et al.*, 1976, Bywater, 1982a, Huber, 1982a, Keefe *et al.*, 1980, Van Miert, 1988). However, there is wide variation in the dose rates (2-20 mg/kg bwt) recommended for parenteral administration of ampicillin to *Equidae* (Bywater, 1982a, Brown *et al.*, 1982, Huber, 1982a, Knight, 1975, Prescott and Baggot, 1988a, Van Miert, 1988). Different preparations of ampicillin may have different pharmacokinetics and, at equimolar dose rates, ampicillin sodium reaches higher concentrations in blood than ampicillin trihydrate and is therefore more likely to produce better clinical results (Tobin, 1979). A number of studies have examined the plasma disposition of ampicillin sodium in horses using a variety of different dose rates (Dürr, 1976, Keefe *et al.*, 1980, Bowman *et al.*, 1986).

Although there have been studies of the disposition of ampicillin following oral administration in other species (Dorrestein *et al.*, 1987, Jusko, 1975, Thompson and Black, 1978, Ziv *et al.*, 1977), there is little information on the oral administration of ampicillin in the equine. However, Brown *et al.* (1984b) have reported serum concentrations of ampicillin in foals following a single oral dose of ampicillin trihydrate.

There have been a number of different studies of the plasma disposition and pharmacokinetics of amoxicillin following intravenous and oral administration. Baggot *et al.* (1988) studied the disposition of amoxicillin following intravenous administration at a dose rate of 20 mg/kg bwt to foals and reported that the disposition of the drug (the apparent volume of distribution and the CL_b, and consequently the elimination half-life) were typical of a penicillin. This suggested that renal function (based upon the CL_b) was adequate in neonatal foals. In man, amoxicillin is absorbed more rapidly and completely from the gastrointestinal tract than ampicillin, and therefore it is more useful clinically (Zarowny *et al.*, 1974). In calves, amoxicillin is absorbed poorly following oral administration, but attains high concentrations in the gastrointestinal tract and would be suitable for treating gastrointestinal infections with susceptible pathogens (Palmer *et al.*, 1977). However, oral

administration of amoxicillin to calves would be unlikely to produce effective serum concentrations against the majority of Gram negative calf pathogens (Ziv and Horsey, 1979). Love *et al.* (1981) reported serum concentrations of amoxicillin following oral administration, at a dose rate of 13-30 mg/kg bwt, to Thoroughbred foals. Peak plasma concentrations, of around 10 μg/ml, were attained within 30-60 min of administration. Although the drug was absorbed adequately (mean systemic availability *circa* 40%) there was wide individual variation in the systemic availability (Baggot *et al.*, 1988). Wilson *et al.* (1988) compared the pharmacokinetics and estimated bioavailability of amoxicillin in mares following intravenous and intramuscular administration (at a dose rate of 10 mg/kg bwt) and oral administration (at a dose rate of 20 mg/kg bwt). Mean peak plasma concentrations of around 3 μg/ml were attained at 1.5 h after oral administration, however the systemic bioavailability was poor (*circa* 10%). Similarly, oral administration of amoxicillin, at a dose rate of 10 mg/kg bwt, was compared with oral administration of pivampicillin (the pivaloyl-oxymethyl ester of ampicillin), at a dose rate of 19.9 mg/kg bwt (equivalent to 15 mg/kg bwt ampicillin), to adult horses (Ensink *et al.*, 1992). Pivampicillin, which is hydrolyzed to ampicillin as it is absorbed from the gastrointestinal tract, had a higher bioavailability than amoxicillin (30% and 5%, respectively) in adult horses (Ensink *et al.*, 1992). Thus, the bioavailability of amoxicillin was low in adult horses and higher in foals.

Generally, the systemic availability of penicillins administered orally is low in the adult horse (Ensink *et al.*, 1992, Wilson *et al.*, 1988) and the risk of disrupting the commensal gastrointestinal microflora is high. That is, assuming that the antimicrobial agent is not destroyed within the gastrointestinal lumen, either by gastric acid or by bacterial metabolism, *e. g.* ß-lactamase production. The extended spectrum penicillins, such as ampicillin, are acid stable and should pass unharmed through the acidic environment of the stomach. In addition, Palmer *et al.* (1977) showed that oral administration of amoxicillin to calves resulted in high drug concentrations throughout the gastrointestinal tract for around 8 h after administration. The advent of inactive prodrugs, such as pivampicillin, which, in theory, are activated following absorption from the gastrointestinal tract, may provide a suitable means of administration of extended spectrum penicillins in the equine.

Ampicillin has not been examined thoroughly with respect to its plasma disposition and pharmacokinetic behaviour in ponies and donkeys. The present study examined the plasma disposition of ampicillin sodium in horses, ponies and donkeys after intravenous administration at a dose rate of 10 mg/kg bwt. Alterations in the intestinal flora were studied by bacteriological examination of serial faecal samples and large intestinal fermentation was studied using faecal SCFA concentrations. The faecal dry matter content and the faecal consistency were used as indicators of the presence or absence of diarrhoea. Similar studies were carried out in ponies with cannulated caecal fistulas following single intravenous and

oral administrations of ampicillin sodium at a dose rate of 10 mg/kg bwt. In addition, drug, bacteriological and SCFA analyses were carried out in caecal liquor, and plasma biochemistry and haematology were monitored.

4.2 Materials and Methods

4.2.1 Intravenous administration of ampicillin to horses, ponies and donkeys

Four Thoroughbred geldings and one mare (No. 1-4 and 6), six ponies (No. 7-9 and 11-13) and three donkeys (No. 15-17) were used as outlined in the general Materials and Methods. Ampicillin sodium was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples were taken prior to intravenous administration of ampicillin and at 0.25, 0.5 0.75, 1, 1.5, 2, 4, 8, 12 and 24 h, with additional samples at 0.033, 0.083 and 6 h in some animals. Faecal samples were taken at 0, 24, and 48 h for drug analysis, bacteriological examination, SCFA analysis and measurement of faecal dry matter content from horses 3, 4 and 6, ponies 7-9 and donkeys 15-17.

4.2.2 Intravenous administration of ampicillin to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 2 occasions (1 and 2). Ampicillin sodium was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis, measurement of pH and SCFA analysis were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h with an additional caecal liquor sample at 48 h. Bacteriological examination of caecal liquor was carried out at 0, 24 and 48 h. Faecal samples were taken for drug analysis, SCFA analysis and measurement of dry matter content at 0, 24 and 48 h. Plasma biochemistry and haematological examinations were carried out at 0, 24 and 48 h.

4.2.3 Oral administration of ampicillin to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 2 occasions (1 and 2) at least 6 weeks after intravenous administration of ampicillin sodium. Ampicillin sodium was administered *via* nasogastric tube at a dose rate of 10 mg/kg bwt. Plasma samples, for drug analysis, and caecal liquor samples, for drug analysis, pH measurement and SCFA analysis, were taken at 0, 0.25, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h with further caecal liquor samples taken at 28, 32, 48, 52, 56, 72, 96 and 168 h. Bacteriological examinations of caecal liquor were carried

out at 0, 24, 48, 72, 96 and 168 h. Faecal samples were taken for measurement of drug concentrations, SCFA concentrations and dry matter content at 0, 24, 48, 72, 96 and 168 h. No plasma biochemistry or haematology samples were taken.

4.2.4 *In vitro* studies with ampicillin

A range of concentrations of ampicillin were incubated for 3 and 24 h in caecal liquor at body temperature in an anaerobic environment, as outlined in the general Materials and Methods, and drug and SCFA concentrations were measured following incubation. A range of concentrations of ampicillin were incubated at pH 1.9 for 1 h at room temperature, as outlined in the general Materials and Methods. A range of concentrations of ampicillin were incubated in the presence of chopped hay at body temperature for 3 h at pH 1.9 and pH 7.0 as outlined in the general Materials and Methods.

4.3 Results of intravenous administration of ampicillin to horses, ponies and donkeys

4.3.1 Plasma disposition and pharmacokinetics

A semilogarithmic plot of plasma concentrations (mean±SEM) following intravenous administration of ampicillin to horses, ponies and donkeys is shown in Figure 4-1. Plasma concentrations from individual animals are given in Appendix B (Tables B1-B3). In all 3 groups, the initial plasma concentrations were similar, however the decline phase of the plasma concentration versus time plot was less steep in horses than it was in ponies and donkeys, which may reflect differences in the elimination of ampicillin. Ampicillin was detected in plasma (>0.02 μg/ml) for up to 8 h in horses and donkeys, and 6 h in ponies.

The pharmacokinetic parameters calculated from the bi-exponential equations used to describe the plasma concentration versus time data are given in Table 4-1, and the individual data is given in Appendix B (Tables B4-B6). The harmonic mean of the elimination and distribution half-lives calculated for horses, ponies and donkeys were similar and quite short. The AUCs in horses and ponies were similar and were larger than the AUC in donkeys. There was a statistically significant difference between the AUC in horses and donkeys ($p = 0.04$). The Vc and Vd were similar in horses and donkeys but were smaller in ponies. This may reflect differences in the extracellular fluid volume or body water compartment. However, it is only a mathematical measure of the how the drug distributes within the body and does not represent actual body compartments. The CL_b was similar in magnitude in horses and ponies and was smaller than in donkeys. There was a statistically significant difference between the CL_b in horses and donkeys ($p = 0.04$).

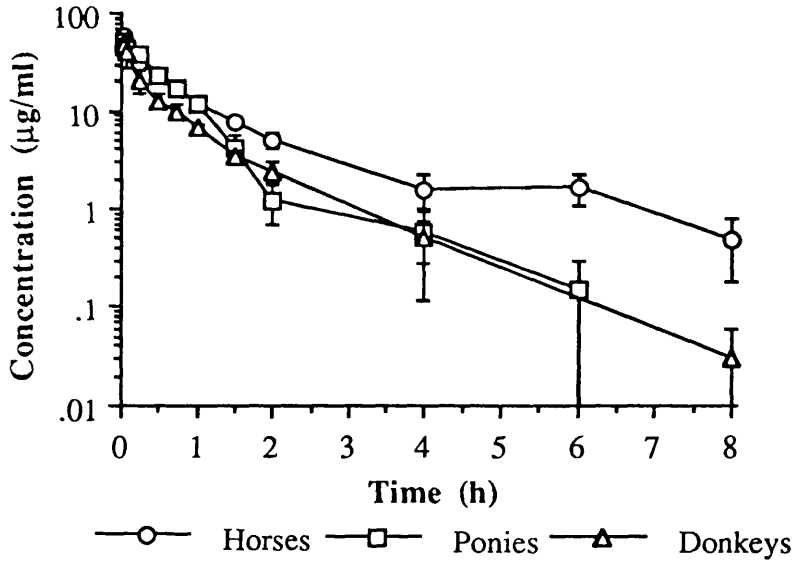

Figure 4-1. Plasma concentrations (mean±SEM) of ampicillin following intravenous administration to horses, ponies and donkeys

Parameter	Horses (n=5)	Ponies (n=6)	Donkeys (n=3)
t1/2 B2 (min)*	14.79	6.62	5.86
t1/2 B1 (min)*	60.88	52.34	42.80
Cp0 (µg/ml)	47.57±8.30	78.35±11.40	55.00±14.81
Vc (ml/kg)	246.19±53.43	152.78±30.97	224.74±80.34
AUC_{obs} (µg.h/ml)	46.70±3.79	46.17±8.07	25.48±3.46
$AUMC_{obs}$ (µg.h^2/ml)	84.72±33.10	55.86±24.05	21.86±3.46
AUC (µg.h/ml)	44.17±2.65	40.38±7.31	24.70±3.06
AUMC (µg.h^2/ml)	73.73±25.86	51.01±18.65	22.00±3.71
MRT (min)	100.07±31.57	63.38±13.29	52.01±7.74
Vd_{area}(ml/kg)	430.05±96.23	386.16±93.22	499.20±172.79
Vd_{ss} (ml/kg)	345.31±85.99	260.44±36.74	422.49±174.80
CL_b (ml/h.kg)	229.67±13.64	257.68±35.14	418.80±56.43
k_{el} (/h)	1.12±0.22	1.97±0.32	2.15±0.44
k_{21} (/h)	1.82±0.79	2.36±0.71	3.23±0.69
k_{12} (/h)	0.56±0.19	2.70±1.52	2.69±0.80

Table 4-1. Disposition kinetics of ampicillin in plasma following intravenous administration to horses, ponies and donkeys

Key: data as mean±SEM; * harmonic mean

4.3.2 Faecal concentrations

There was no ampicillin detected in faeces following intravenous administration to horses, ponies and donkeys.

4.3.3 Bacteriological examinations

Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of ampicillin are shown in Figures 4-2, 4-3 and 4-4 and the individual data is given in Appendix B (Tables B7-B9).

Salmonella spp. and *C. difficile* were selected for but not isolated.

In the horses, the mean number of coliforms isolated was high (10^9/g) at 48 h after drug administration (Figure 4-2). However, there were no coliforms isolated from horse 3 at 0 and 48 h, no marked alterations in the number of coliforms isolated from horse 4, and a high number of coliforms (10^{10}/g) isolated from horse 6 at 48 h after drug administration. The mean number of streptococci isolated from horses was high (10^8/g) at 48 h after drug administration. There was an increase in the number of streptococci isolated at 48 h (10^8/g) after drug administration to horse 6. There were no marked alterations in the number of lactobacilli, *Bacteroides spp.*, or *Clostridium spp.* isolated from horses following intravenous administration of ampicillin. However, the number of *Bacteroides spp.* isolated from horses was low (10^6-10^8/g) on all occasions, except from horse 4 (10^9/g) prior to drug administration.

There was a slight increase (to 10^7/g) in the mean number of coliforms isolated from ponies at 24 and 48 h after drug administration (Figure 4-3). There was an increase (to 10^8/g) in the number of coliforms isolated from pony 8 at 24 h, and from pony 7 at 24 and 48 h after drug administration. There was a slight increase (to 10^7/g) in the mean number of streptococci isolated from ponies at 24 and 48 h after drug administration. There was an increase in the number of streptococci isolated from pony 7 at 24 h (10^8/g) and from pony 8 at 48 h (10^8/g). There were no marked alterations in the number of lactobacilli, *Clostridium spp.* or *Bacteroides spp.* isolated following drug administration. Although, there were low numbers of *Bacteroides spp.* (<10^9/g) isolated at all times, except at 24 h from pony 7 and 48 h from pony 8. There were moderately high numbers of *Clostridium spp.* isolated from pony 7 (10^6/g) at 24 h and from pony 9 (10^6/g) at 48 h.

There was a slight increase (to 10^7/g) in the mean number of coliforms isolated from donkeys at 48 h after drug administration (Figure 4-4). This was due to an increase in the number of coliforms isolated from donkey 16 at 48 h (10^8/g). The mean number of

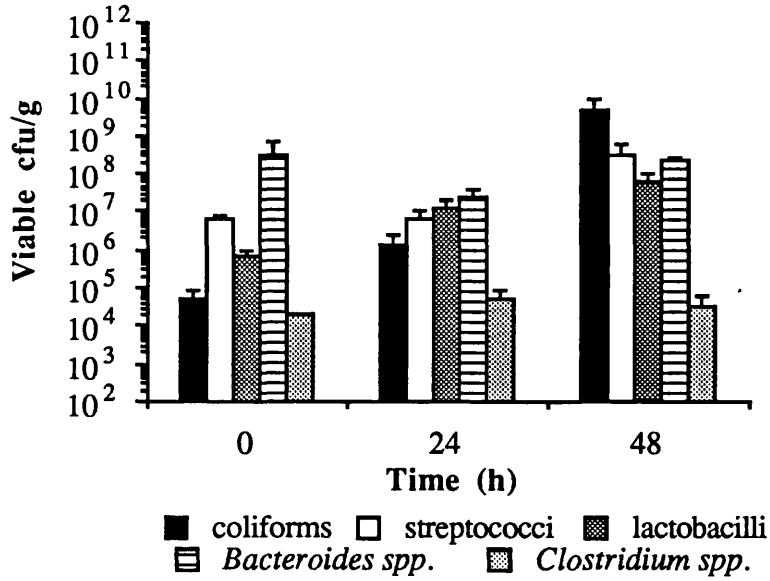

Figure 4-2. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of ampicillin to horses

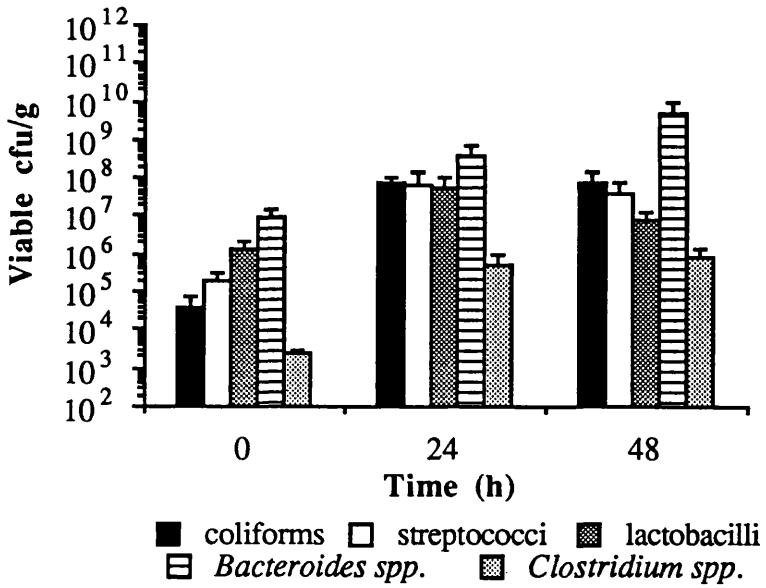

Figure 4-3. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of ampicillin to ponies

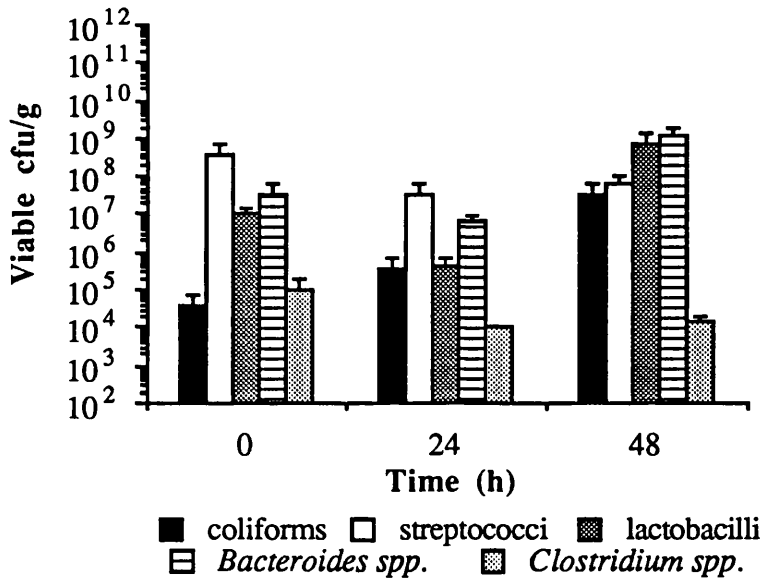

Figure 4-4. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of ampicillin to donkeys

streptococci isolated was high (10^7-10^8/g) throughout the study. There were high numbers (10^8-10^9/g) of streptococci isolated from donkey 15 at 0, 24 and 48 h, from donkeys 16 and 17 prior to drug administration, and from donkey 16 at 48 h. There was a slight increase (to 10^8/g) in the mean number of lactobacilli isolated at 48 h after drug administration to donkeys. There were high numbers (10^8-10^9/g) of lactobacilli isolated from donkeys 15 and 17 at 48 h after drug administration. There were no marked alterations in the number of *Bacteroides spp.* or *Clostridium spp.* isolated following intravenous administration of ampicillin to donkeys. However, the number of *Bacteroides spp.* isolated was low throughout the study (10^6-10^8/g), except from donkeys 16 and 17 (10^9/g) at 48 h.

4.3.4 Faecal SCFA concentrations

Faecal lactic acid and total VFA concentrations (mean ±SEM) following intravenous administration of ampicillin to horses, ponies and donkeys are shown in Figures 4-5 and 4-6, and the individual and mean data are given in Appendix B (Tables B10-B19). The SCFA concentrations in faeces of horses, ponies and donkeys were very variable. Lactic acid concentrations in faeces remained within the normal range of 0.0-24.4 mmol/l (Figure 4-5).

There were marked variations in faecal total and individual VFA concentrations in horses, ponies and donkeys following intravenous administration of ampicillin (Appendix B, Tables B10-B18). In horses, mean total VFA concentrations were below the normal range of 24.4-109.2 mmol/l at 0, 24 and 48 h after intravenous administration of ampicillin. In ponies, mean total VFA concentrations were within the normal range prior to drug administration, and lower than normal at 24 and 48 h after intravenous administration of ampicillin. In donkeys, the mean total VFA concentrations were within the normal range at 0, 24 and 48 h. The variations in total and individual VFA concentrations were considered not to be associated with intravenous administration of ampicillin.

4.3.5 Faecal dry matter content and consistency

A plot of faecal dry matter content (mean±SEM) versus time following intravenous administration of ampicillin to horses, ponies and donkeys is shown in Figure 4-7, and the individual data is given in Appendix B (Tables B20-B22). The mean faecal dry matter content was reduced slightly (*circa* 17%) in horses at 24 h after ampicillin administration. Faecal dry matter content was low (<17%) at 24 h in horses 4 and 6, and at 48 h in horse 6. There were no marked alterations in the mean faecal dry matter content following intravenous administration of ampicillin to ponies and donkeys. The faecal dry matter content was low (*circa* 13%) in donkey 17 prior to drug administration. No alterations in faecal consistency were observed following intravenous administration of ampicillin to

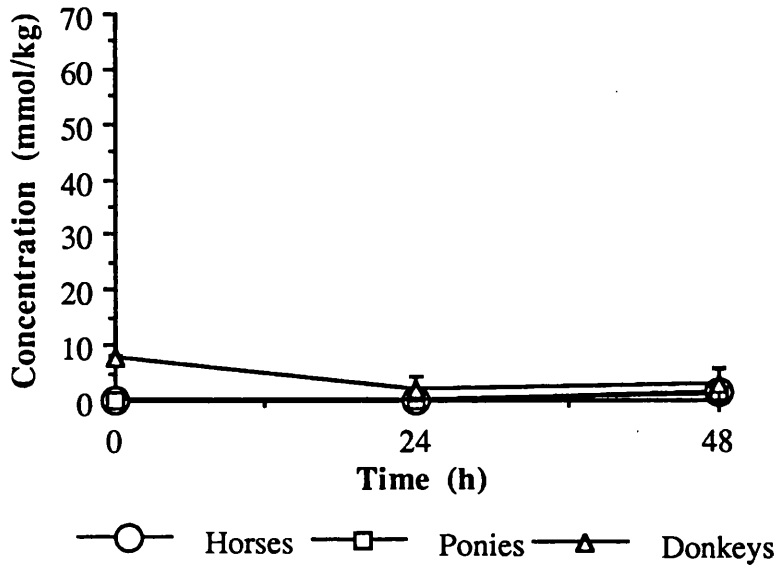

Figure 4-5. Lactic acid concentrations (mean±SEM) in faeces following intravenous administration of ampicillin to horses, ponies and donkeys

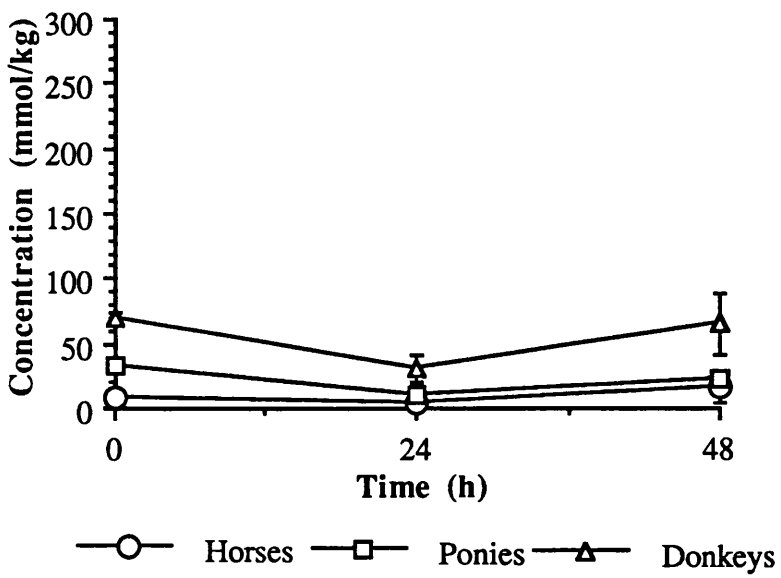

Figure 4-6. Total VFA concentrations (mean±SEM) in faeces following intravenous administration of ampicillin to horses, ponies and donkeys

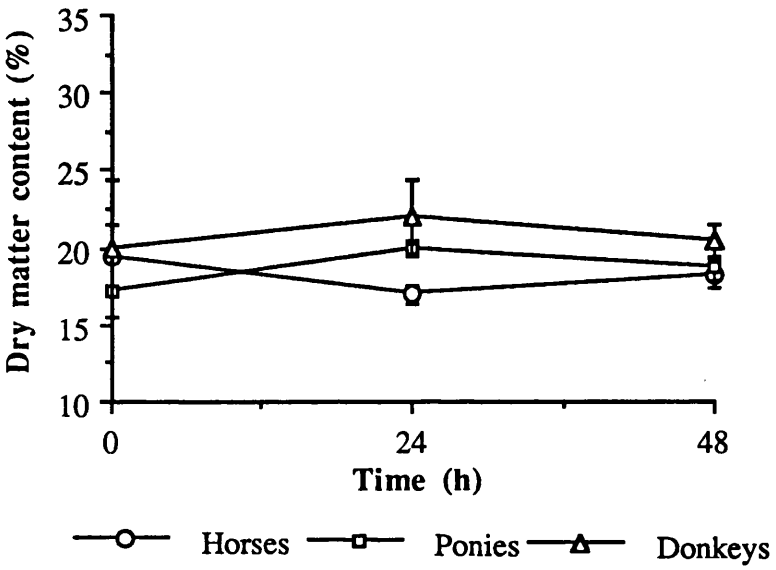

Figure 4-7. Faecal dry matter content (mean±SEM) following intravenous administration of ampicillin to horses, ponies and donkeys

horses, ponies and donkeys. Although, faecal consistency was soft prior to drug administration to donkey 17.

4.4 Results of intravenous administration of ampicillin to ponies with cannulated caecal fistulas

4.4.1 Plasma disposition and pharmacokinetics

The plasma concentrations of ampicillin following intravenous administration to ponies I and II on occasions 1 and 2 are given in Appendix B (Table B23).

A bi-exponential equation best described the data from ponies I1 and II2. The plasma concentration versus time data from pony I2 and II1 could not be described using a bi-exponential equation since it was distributed very rapidly and the disposition curve was described by a one-compartment model (elimination phase only). The pharmacokinetic parameters calculated from the plasma concentration versus time data of each animal on each occasion are given in Table 4-2.

4.4.2 Caecal liquor and faecal concentrations

The caecal liquor concentrations of ampicillin following intravenous administration to ponies I and II on occasions 1 and 2 are given in Appendix B (Table B24). No ampicillin was detected in caecal liquor from ponies II1 or II2 following intravenous administration. The caecal liquor concentrations of ampicillin versus time from pony I are shown in Figure 4-8. Maximum caecal liquor concentrations were 2.26 and 1.13 μg/ml at 1.5 and 0.25 h on occasions 1 and 2, respectively. The caecal AUC was expressed as a percentage of the intravenous AUC, taking into account the estimated volume of caecal contents of 6.67 l in pony I1 and the estimated plasma volume of 51 ml/kg bwt (Swenson, 1977) or 11.88 l, and was 3.66% in pony I1 and 3.18% in pony I2.

Drug disposition within the caecum was described using AUC and AUMC for observed values and the ratio of these (MRT). The pharmacokinetic variables that were calculated are given in Table 4-3.

There was no ampicillin detected in faecal samples following intravenous administration to ponies.

Parameter	I1§	I2*	II1*	II2§
t1/2 B2 (min)	3.42	-	-	40.74
t1/2 B1 (min)	70.62	87.78	101.82	144.30
Cp0 (μg/ml)	118.36	20.57	21.31	17.70
Vc (ml/kg)	84.50	486.17	469.28	565.01
AUC_{obs} (μg.h/ml)	54.77	45.61	53.01	52.24
$AUMC_{obs}$ (μg.h^2/ml)	73.21	90.90	119.89	171.63
AUC (μg.h/ml)	48.21	43.43	52.18	50.55
AUMC (μg.h^2/ml)	69.30	91.70	127.79	164.77
MRT (min)	80.20	119.58	135.70	197.12
Vd_{area} (ml/kg)	352.30	486.17	469.28	686.70
Vd_{SS} (ml/kg)	298.18	486.17	469.28	644.95
CL_b (ml/h.kg)	207.43	230.26	191.63	197.84
k_{el} (/h)	2.46	0.47	0.41	0.35
k_{21} (/h)	2.91	-	-	0.84
k_{12} (/h)	7.74	-	-	0.12

Table 4-2. Disposition kinetics of ampicillin in plasma following intravenous administration to ponies

Key: * calculated from a mono-exponential equation;§ calculated from a bi-exponential equation

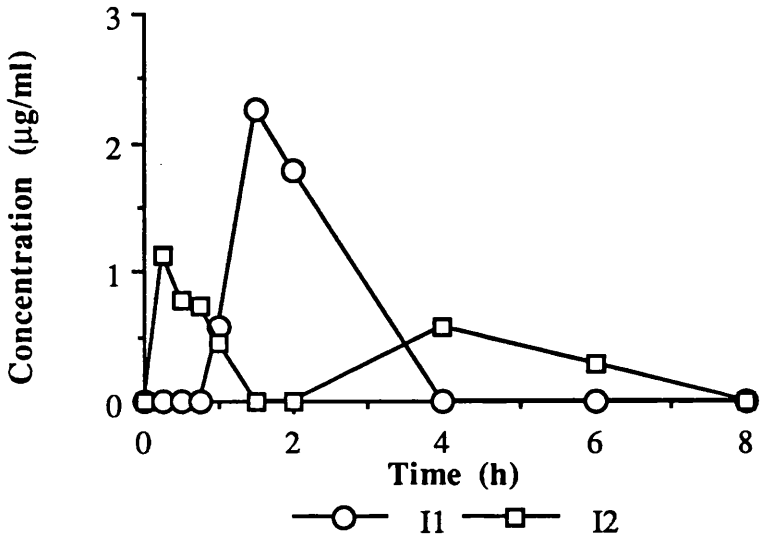

Figure 4-8. Caecal liquor concentrations of ampicillin following intravenous administration to ponies I1 and I2

Parameter	I1	I2
AUC_{obs} (µg.h/ml)	3.57	2.58
$AUMC_{obs}$ (µg.h^2/ml)	6.36	8.60
MRT (h)	1.78	3.34

Table 4-3. Disposition kinetics of ampicillin in caecal liquor following intravenous administration to pony I

4.4.3. Bacteriological examinations

Counts of viable bacteria in caecal liquor following intravenous administration of ampicillin are shown in Figures 4-9 to 4-13, and the individual data is given in Appendix B (Table B25).

Salmonella spp. and *C. difficile* were selected for but not isolated.

There were no marked alterations in the number of coliforms (Figure 4-9), lactobacilli (Figure 4-11), *Bacteroides spp.* (Figure 4-12) or *Clostridium spp.* (Figure 4-13) isolated from ponies following intravenous administration of ampicillin. There was an increase in the number of streptococci (up to 10^8/ml) isolated from pony I1 at 24 h, and from pony II1 at 48 h after intravenous administration of ampicillin (Figure 4-10). Otherwise there were no marked alterations in the number of streptococci isolated. The number of *Bacteroides spp.* isolated was low (10^7-10^8/ml) at 48 h in pony I1, at 24 and 48 h in pony I2, and at 24 h in ponies II1 and II2. *Clostridium clostridiiforme* was isolated and identified using the API system.

4.4.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

Caecal liquor pH values following intravenous administration of ampicillin are shown in Figure 4-14, and the individual data is given in Appendix B (Table B26). The caecal liquor pH was slightly high (7.3-7.5) at 0.25, 0.75, 1, 1.5, 2, 4 and 6 h after drug administration to pony I1. In pony I2, caecal liquor pH was high (7.3-7.8) at 0.25, 0.5, 0.75, 1, 1.5, 2 and 8 h after drug administration. In pony II1, caecal liquor pH was low (6.6-6.7) at 0, 0.25 and 12 h after drug administration and slightly high (7.3-7.5) at 2 and 48 h after drug administration. In pony II2, caecal liquor pH was lower than normal (6.4-6.7) at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 24 and 48 h following intravenous administration of ampicillin.

Lactic acid concentrations in caecal liquor following intravenous administration of ampicillin to ponies are shown in Figure 4-15, and the individual data is given in Appendix B (Tables B27a-B30a). In pony I1, lactic acid concentrations were increased to 10.1-43.6 mmol/l at 4, 6, 8, 12 and 24 h after drug administration and outside the normal range of 0.0-24.4 mmol/l at 6, 8, and 12 h after drug administration. In pony I2, lactic acid concentrations were increased to 9.5-44.4 mmol/l at 8, 12 and 24 h after drug administration and were outside the normal range at 24 h after drug administration. In ponies II1 and II2, caecal lactic acid concentrations were within the normal range at all times sampled.

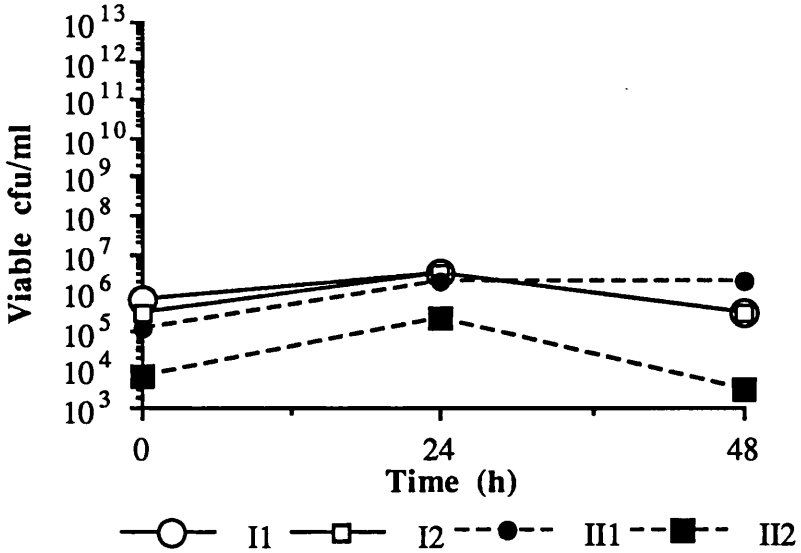

Figure 4-9. Counts of viable coliforms in caecal liquor following intravenous administration of ampicillin to ponies

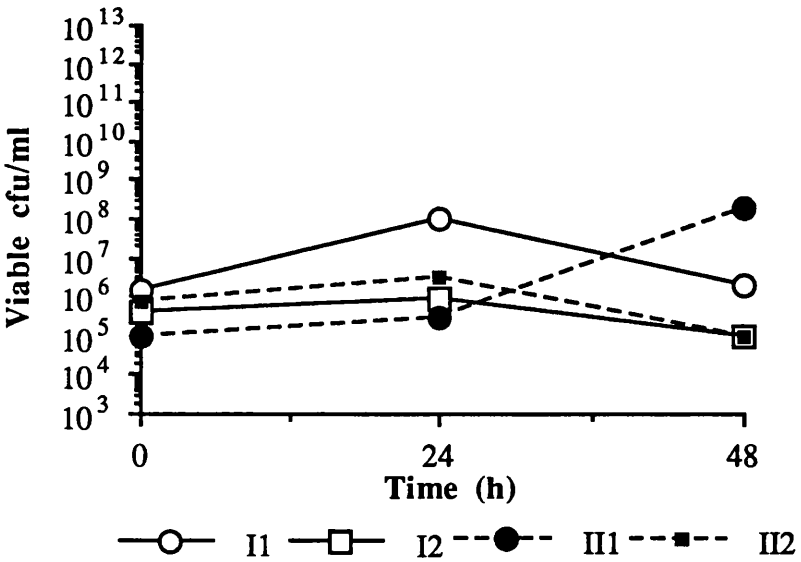

Figure 4-10. Counts of viable streptococci in caecal liquor following intravenous administration of ampicillin to ponies

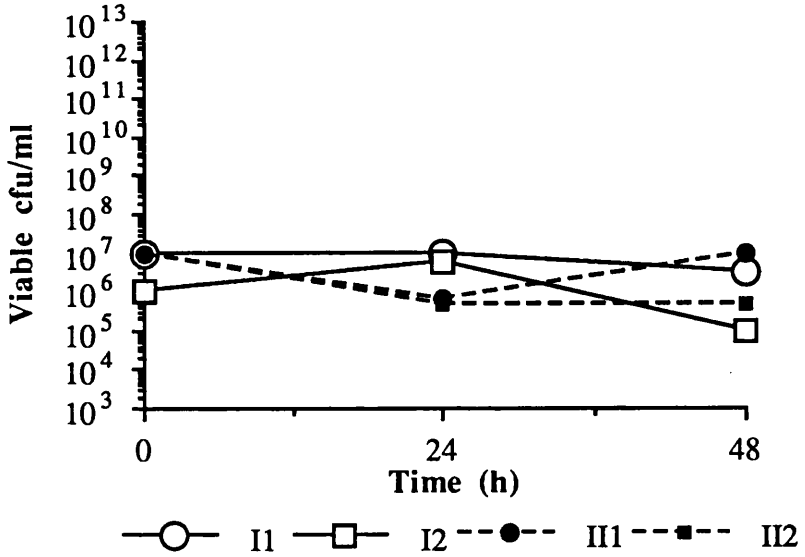

Figure 4-11. Counts of viable lactobacilli in caecal liquor following intravenous administration of ampicillin to ponies

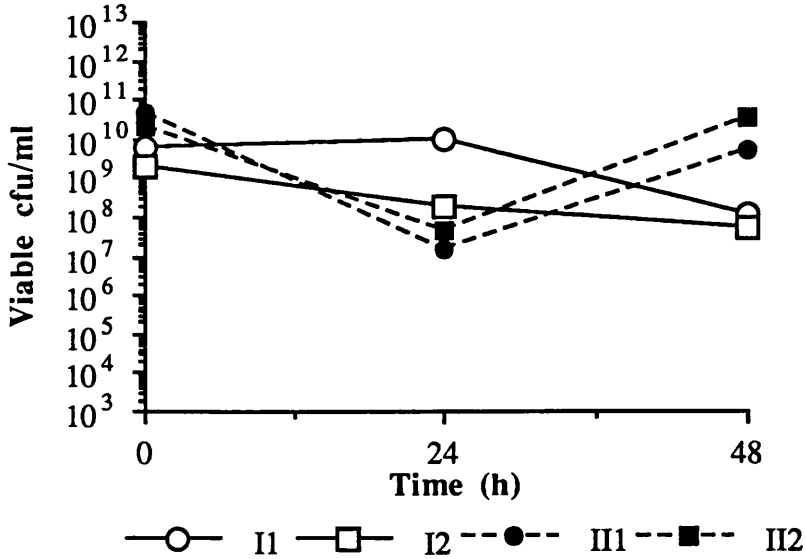

Figure 4-12. Counts of viable *Bacteroides spp.* in caecal liquor following intravenous administration of ampicillin to ponies

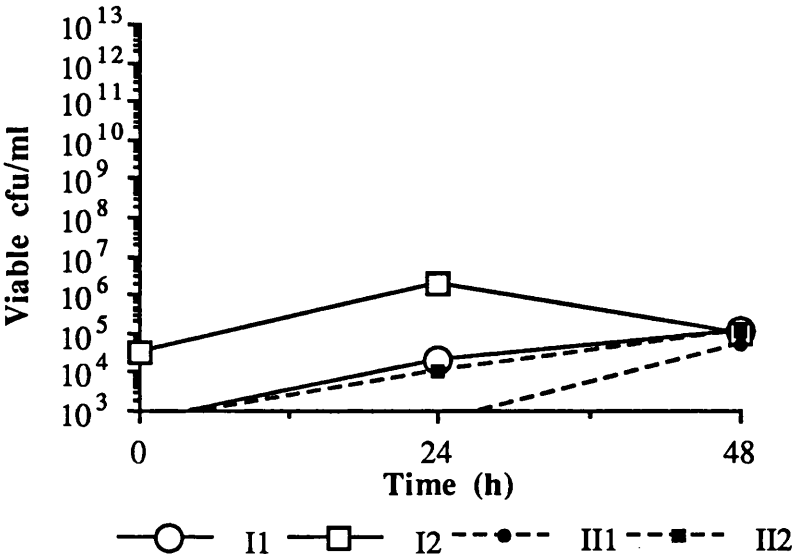

Figure 4-13. Counts of viable *Clostridium spp.* in caecal liquor following intravenous administration of ampicillin to ponies

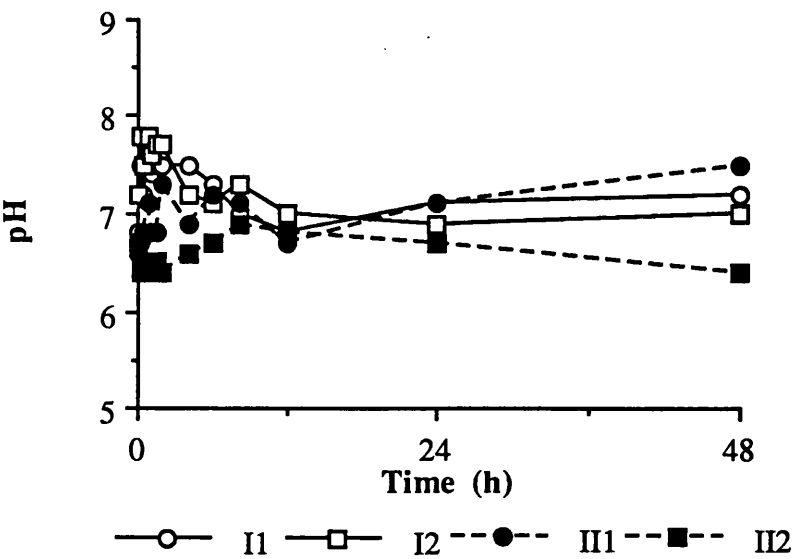

Figure 4-14. Caecal liquor pH following intravenous administration of ampicillin to ponies

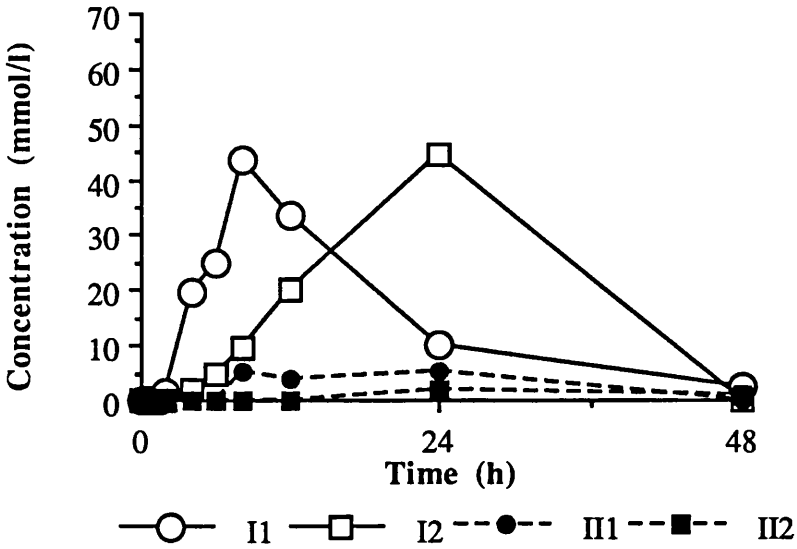

Figure 4-15. Lactic acid concentrations in caecal liquor following intravenous administration of ampicillin to ponies

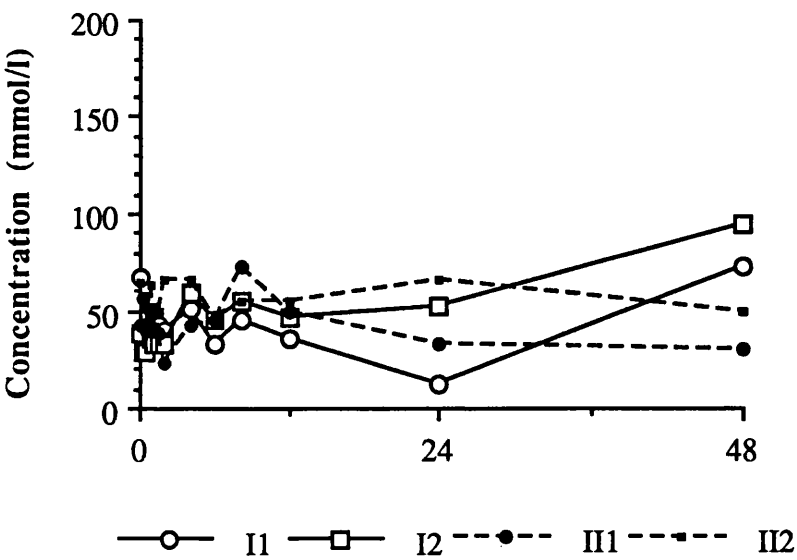

Figure 4-16. Total VFA concentrations in caecal liquor following intravenous administration of ampicillin to ponies

Caecal liquor total VFA concentrations are shown in Figure 4-16, and the total VFA and individual acid concentrations are given in Appendix B (Tables B27a-B30a). There were fluctuations in the total and individual VFA concentrations around the normal range. In pony I1, the caecal liquor total VFA concentration of 12.5 mmol/l was lower than the normal range of 24.4-109.2 mmol/l at 24 h after intravenous administration of ampicillin. In addition, acetic (8.4-11.0 mmol/l) and butyric (0.0 mmol/l) acid concentrations were lower than the normal ranges of 12.6-64.5 and 4.8-67.3 mmol/l at 6, 8 and 24 h, and 0.25 and 24 h, respectively. In ponies I2, II1 and II2, total VFA and individual VFA concentrations were all within or around the normal ranges following intravenous administration of ampicillin.

The proportions of acetic, propionic and butyric acids in pony I1, as a percentage of total VFA concentrations, are given in Appendix B (Table B27b). The percentage of acetic acid was increased to 80.6%, and was above the normal range of 27.9-67.0%, at 0.25 h after drug administration. There was an increase in the percentage of propionic acid to 56.9%, above the normal range of 4.9-38.5%, at 8 h after drug administration. There was no butyric acid detected at 0.25 and 24 h after drug administration. There was a reduction in the percentage of propionic plus butyric acid to 19.4% (normal 27.1-72.1%) at 0.25 h and an increase to 73.6-77.7% at 6 and 8 h after drug administration. The ratios of acetic, propionic and butyric acid concentrations in ponies I2, II1 and II2 are given in Appendix B (Table B28b, B29b, B30b) and were all within or around the normal ranges.

Lactic acid concentrations in faeces were within the normal range of 0.0-24.4 mmol/l at all sample times in all ponies, and the individual data is given in Appendix B (Tables B31-B34). Total VFA concentrations in faeces are shown in Figure 4-17, and the individual data is given in Appendix B (Tables B31-B34). The total VFA concentrations in faeces were lower than normal range of 24.4-109.2 mmol/l prior to drug administration in pony I1, at 48 h in pony I2, and throughout the study in pony II2. In pony II1, total VFA concentrations were within the normal range throughout the study. There were considerable fluctuations in individual VFA concentrations but none of these were attributed to the intravenous administration of ampicillin.

4.4.5 Faecal dry matter content and consistency

The faecal dry matter content following intravenous administration of ampicillin to ponies is shown in Figure 4-18, and the individual data is given in Appendix B (Table B35). There were no marked alterations in the faecal dry matter content following drug administration. The faecal dry matter content was high (*circa* 33%) at 0 and 24 h in pony I2. No changes in faecal consistency were observed.

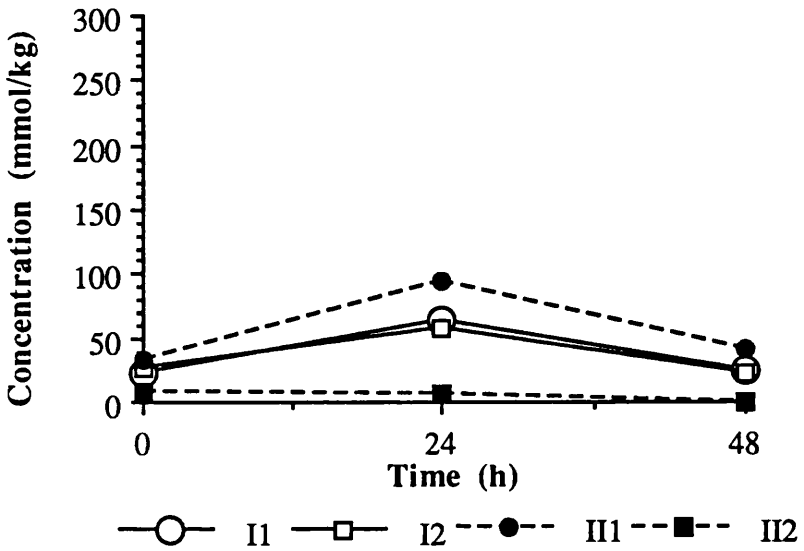

Figure 4-17. Total VFA concentrations in faeces following intravenous administration of ampicillin to ponies

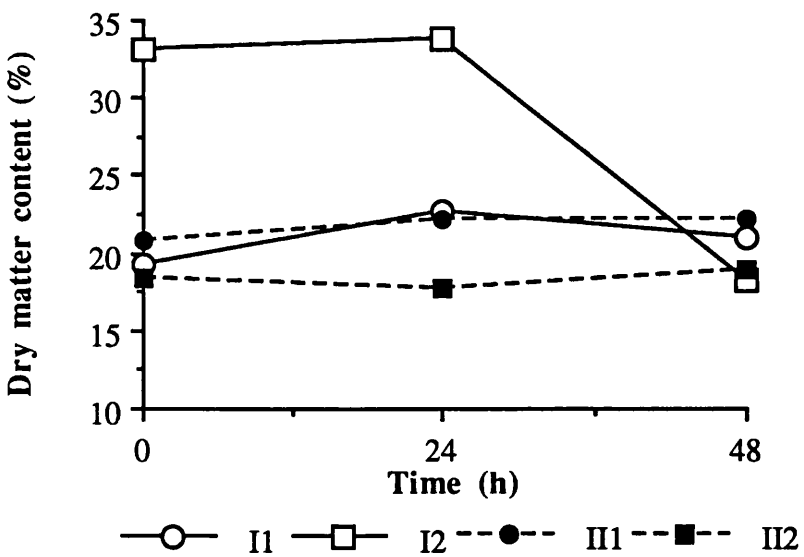

Figure 4-18. Faecal dry matter content following intravenous administration of ampicillin to ponies

4.4.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix B (Tables B36 and B37) and the results of the haematological examinations are given in Appendix B (Tables B38 and B39). There were no alterations in plasma biochemistry or in haematology that were considered to be associated with the intravenous administration of ampicillin.

4.5 Results of oral administration of ampicillin to ponies with cannulated caecal fistulas

4.5.1 Plasma disposition and pharmacokinetics

The plasma concentrations of ampicillin following oral administration to ponies are shown in Figure 4-19, and the individual data is given in Appendix B (Table B40). Maximum plasma concentrations of 2.30, 1.16, 1.96 and 0.74 μg/ml were measured at 0.5 h in ponies I1 and I2, 0.75 h in pony II1, and 0.5 h in pony II2, respectively.

The pharmacokinetic parameters calculated following oral administration of ampicillin to ponies are given in Table 4-4. A lag time of 8.82 min was required to describe the plasma concentration versus time data following oral administration of ampicillin to pony II1. The MAT was calculated as the difference between the mean MRT, following intravenous administration to each animal and the MRT following oral administration on each occasion. The systemic availability of ampicillin was calculated from the observed AUC_{oral} and the mean observed $AUC_{intravenous}$ was low (1-6%) following oral administration.

4.5.2 Caecal liquor and faecal concentrations

The caecal liquor concentrations following oral administration of ampicillin to ponies are shown in Figure 4-20, and the individual data is given in Appendix B (Table B41). Maximum caecal liquor concentrations of 101.31, 144.53, 47.40 and 3.63 μg/ml were measured at 2 h in ponies I1 and I2, and 6 h in ponies II1 and II2, respectively. The caecal concentrations were much higher than the plasma concentrations of ampicillin following oral administration.

The disposition of ampicillin in the caecal liquor was described using observed AUC and AUMC to calculate MRT (Table 4-5). The AUC, AUMC and MRT were much larger than those calculated for ampicillin in plasma following oral administration. There was a marked difference in MRT between the 2 animals, a fact that was also reflected in the difference in the time to maximum caecal liquor concentrations.

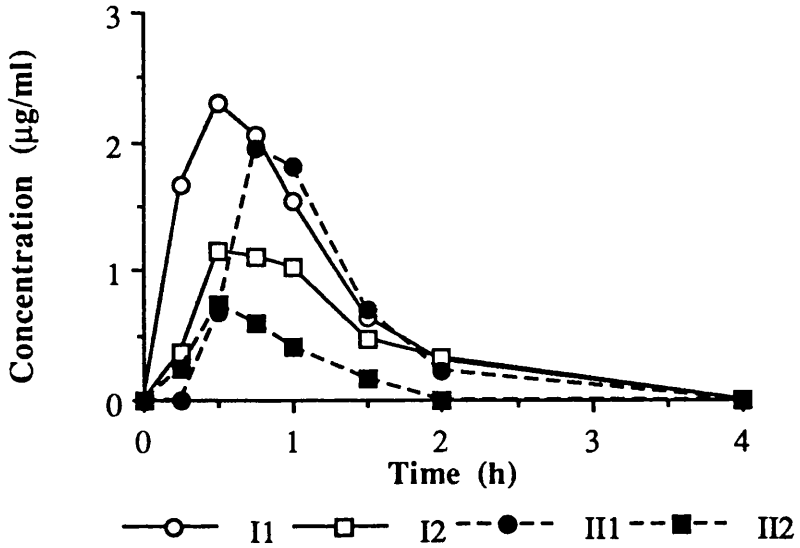

Figure 4-19. Plasma concentrations following oral administration of ampicillin to ponies

Parameter	I1	I2	II1	II2
t1/2 B2 (min)	10.44	10.50	12.54	8.76
t1/2 B1 (min)	26.40	39.78	20.16	23.52
C_{max} (µg/ml)	2.30	1.16	1.96	0.74
t_{max} (min)	30	30	45	30
AUC_{obs} (µg.h/ml)	2.78	1.69	1.97	0.63
$AUMC_{obs}$ (µg.h^2/ml)	2.59	1.94	2.22	0.50
AUC (µg.h/ml)	2.68	1.69	1.97	0.73
AUMC (µg.h^2/ml)	2.38	2.04	1.55	0.57
MRT (min)	55.90	68.88	67.61	47.62
MAT (min)	-43.99	-31.01	-98.80	-118.79
F (%)	5.54	3.37	3.74	1.20

Table 4-4. Disposition kinetics of ampicillin in plasma following oral administration to ponies

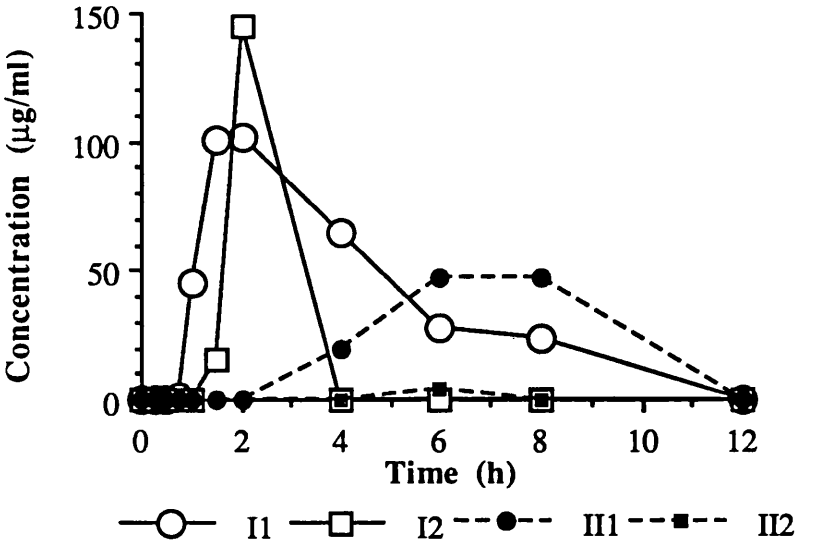

Figure 4-20. Caecal concentrations following oral administration of ampicillin to ponies

Parameter	I1	I2	II1	II2
AUC_{obs} (µg.h/ml)	450.82	188.18	273.81	8.37
$AUMC_{obs}$ (µg.h^2/ml)	1767.05	372.60	1848.00	52.44
MRT (h)	3.92	1.98	6.75	6.27

Table 4-5. Disposition kinetics of ampicillin in caecal liquor following oral administration to ponies

No ampicillin was detected in faecal samples following oral administration to ponies I and II on occasions 1 and 2.

4.5.3 Bacteriological examinations

Salmonella spp. and *C. difficile* were selected for but not isolated.

Counts of viable bacteria in caecal liquor following oral administration of ampicillin are shown in Figures 4-21 to 4-25, and the individual data is given in Appendix B (Table B42).

There was an increase (to 10^8-10^{11}/ml) in the number of viable coliforms isolated following oral administration of ampicillin to ponies (Figure 4-21). However, in pony I1, the initial number of coliforms isolated was high (10^{11}/ml) and it remained so until 72 h after drug administration. In pony I2, there was a slight increase in the number of coliforms isolated at 24 and 96 h after drug administration (10^7-10^8/ml). In ponies II1 and II2, the number of coliforms isolated was increased to 10^9-10^{11}/ml at 24 and 48 h following oral administration of ampicillin.

There was an increase (to 10^8-10^{10}/ml) in the number of viable streptococci isolated following oral administration of ampicillin to ponies (Figure 4-22). However, in pony I1 there were high numbers of streptococci (10^8-10^{10}/ml) isolated initially and up until 96 h after drug administration. In pony I2, there was a slight increase (up to 10^7/ml) in the numbers of streptococci isolated at 24 and 48 h after drug administration. In pony II1, there were high numbers (up to 10^{10}/ml) of streptococci isolated at 48 and 72 h after oral administration of ampicillin. In pony II2, there was an increase (to 10^8-10^9/ml) in the number of streptococci isolated at 48 and 72 h after drug administration, and a low number (10^3/ml) of streptococci isolated at 168 h.

There was an apparent increase in the number of viable lactobacilli isolated from caecal liquor following oral administration of ampicillin to ponies (Figure 4-23). There were high numbers of lactobacilli (10^9-10^{11}/ml) isolated from pony I1 at 0, 24 and 48 h, from pony I2 at 24 h, and from ponies II1 and II2 at 0 and 48 h. *Lactobacillus acidophilus* was isolated and identified using the API system.

There were no marked alterations in the number of viable *Bacteroides spp.* isolated following oral administration of ampicillin to ponies (Figure 4-24). However, there were low numbers of *Bacteroides spp.* (10^7-10^8/ml) isolated from pony I1 at 72 and 168 h, from pony I2 throughout the study, from pony II1 at 0, 72 and 168 h, and from pony II2 at 0 and 24 h. *Bacteroides spp.* identified using the API system were *B. capillosus*, *B. diastonis*, *B. oralis*, *B. ovatus* and *B. thetaiotaomicron*.

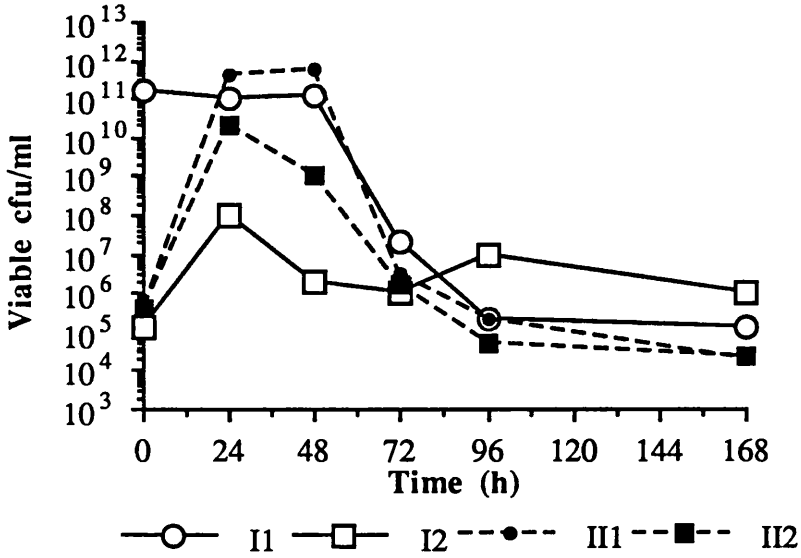

Figure 4-21. Counts of viable coliforms in caecal liquor following oral administration of ampicillin to ponies

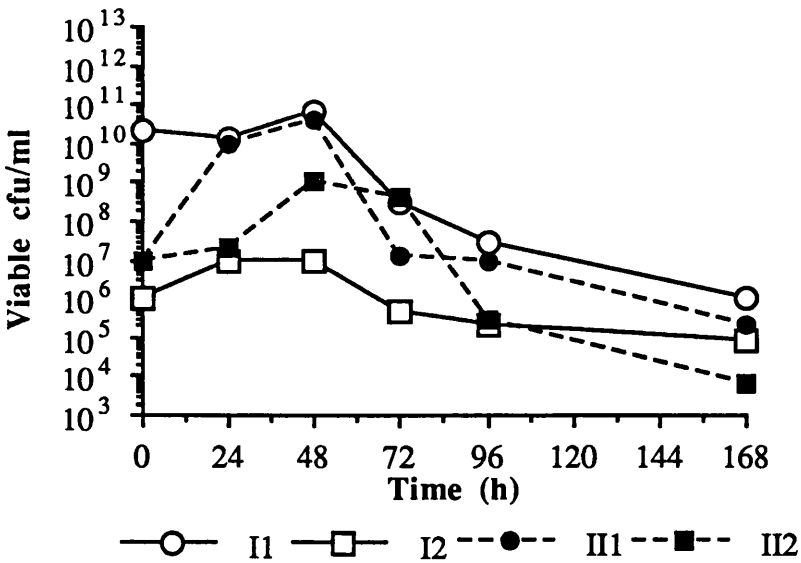

Figure 4-22. Counts of viable streptococci in caecal liquor following oral administration of ampicillin to ponies

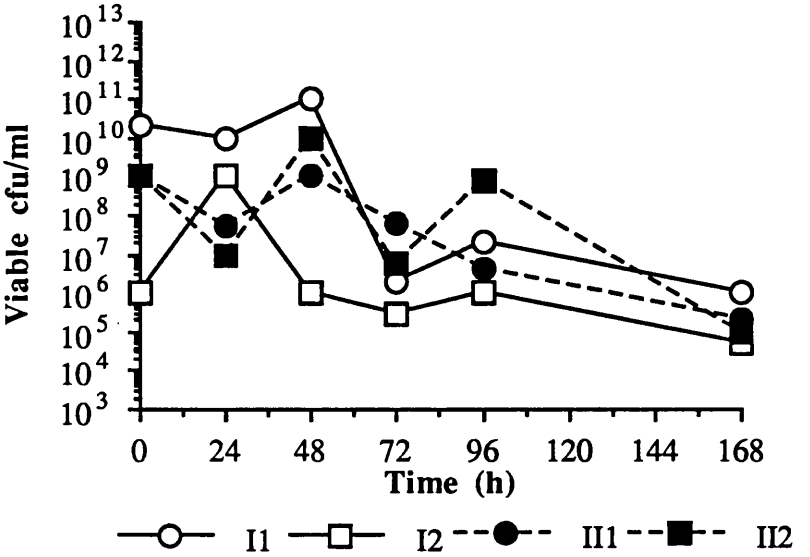

Figure 4-23. Counts of viable lactobacilli in caecal liquor following oral administration of ampicillin to ponies

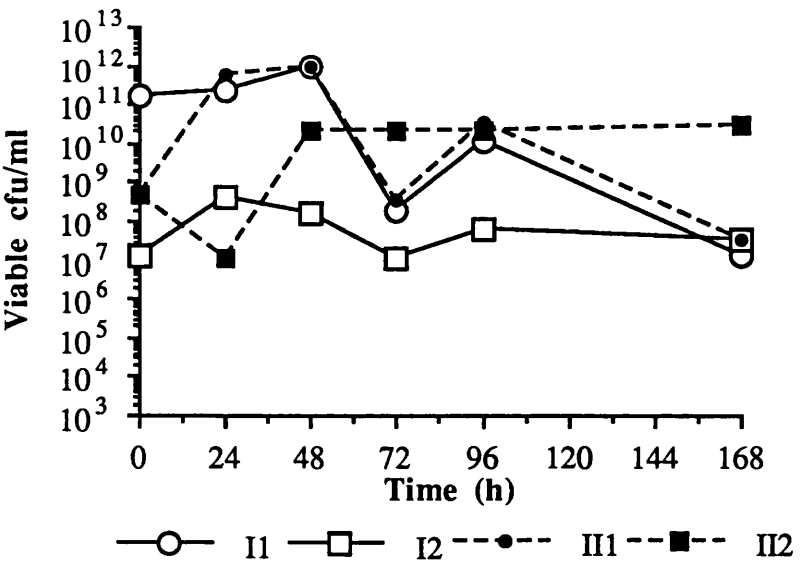

Figure 4-24. Counts of viable *Bacteroides spp.* in caecal liquor following oral administration of ampicillin to ponies

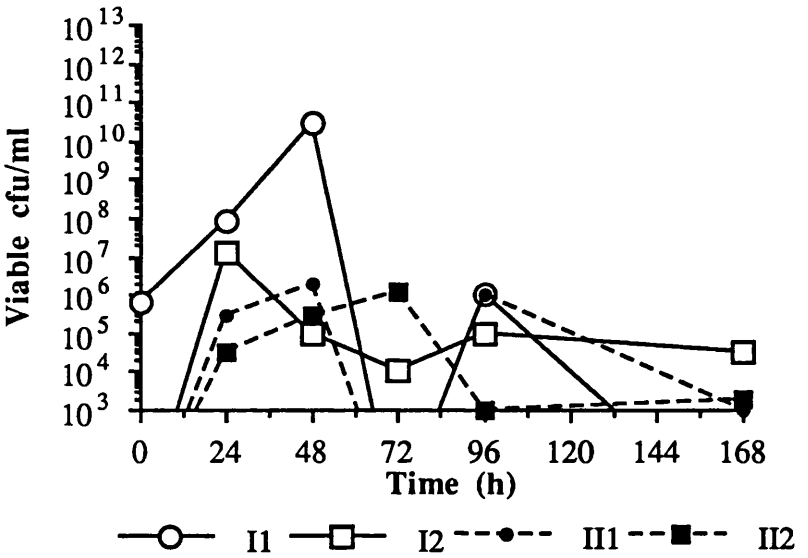

Figure 4-25. Counts of viable *Clostridium spp.* in caecal liquor following oral administration of ampicillin to ponies

There was an apparent increase in the number of *Clostridium spp.* isolated from caecal liquor following oral administration of ampicillin (Figure 4-25). There was an increase in the number of *Clostridium spp.* isolated from pony I1 at 24 and 48 h (10^7-10^{10}/ml), and from pony I2 at 24 h (10^7/ml). The increase in the number of *Clostridium spp.* isolated from ponies II1 and II2 was less marked. There was an increase in the numbers of *Clostridium spp.* isolated from pony II1 at 48 and 96 h (10^6/ml) and from pony II2 at 72 h (10^6/ml). The *Clostridium spp.* identified using the API system were *C. butyricum* and *C. ramosum.*

In addition, *Peptococcus spp.* was isolated and identified using the API system.

4.5.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

The caecal liquor pH measurements are shown in Figure 4-26, and the individual data is given in Appendix B (Table B43). There were increases and decreases in caecal liquor pH measured following oral administration of ampicillin to ponies. In pony I1, the caecal liquor pH was increased (up to a maximum of 7.5) at 6, 8, and 12 h and decreased (down to a minimum of 6.4) at 1.5, 24, 72 and 168 h after drug administration. In pony I2, the caecal liquor pH was increased (up to a maximum of 8.1) at 4, 6, 8, 24, 96 and 168 h and decreased (down to a minimum of 5.7) at 0, 12, 28, 32 and 72 h after drug administration. There was an increase in the caecal liquor pH in pony II1 at 72 h (7.3) and a decrease at 168 h (6.7) after drug administration. There was an increase in the caecal liquor pH (up to a maximum of 7.5) at 6, 24, 32, 96 and 168 h and a decrease (down to a minimum of 6.6) at 0, 0.25, 0.5, 0.75, 1 and 12 h after drug administration to pony II2.

Caecal liquor SCFA concentrations are shown in Figures 4-27 and 4-28, and the individual data is given in Appendix B (Tables B44a-B47a).

Caecal liquor lactic acid concentrations increased following oral administration of ampicillin to ponies (Figure 4-27). In pony I1, lactic acid concentrations were increased to 9.8-42.7 mmol/l at 4, 6, 8, 12, 24, 28 and 32 h, and were above the normal range of 0.0-24.4 mmol/l at 8, 12, 24, 28 and 32 h after drug administration. Caecal liquor lactic acid concentrations were elevated to 6.5-51.6 mmol/l in pony I2 at 4, 8, 12, 32 and 48 h and were outside the normal range at 6 and 8 h after drug administration. In pony II1, caecal liquor lactic acid concentrations were increased to 5.7-39.6 mmol/l at 8, 12, 24, 28 and 32 h and were outside the normal range at 24, 28 and 32 h after drug administration. Similarly, in pony II2 lactic acid concentrations in caecal liquor were elevated to 6.3-40.5 mmol/l at 8, 12, 24, 28, 32 and 48 h, and outside the normal range at 12 and 24 h after drug administration.

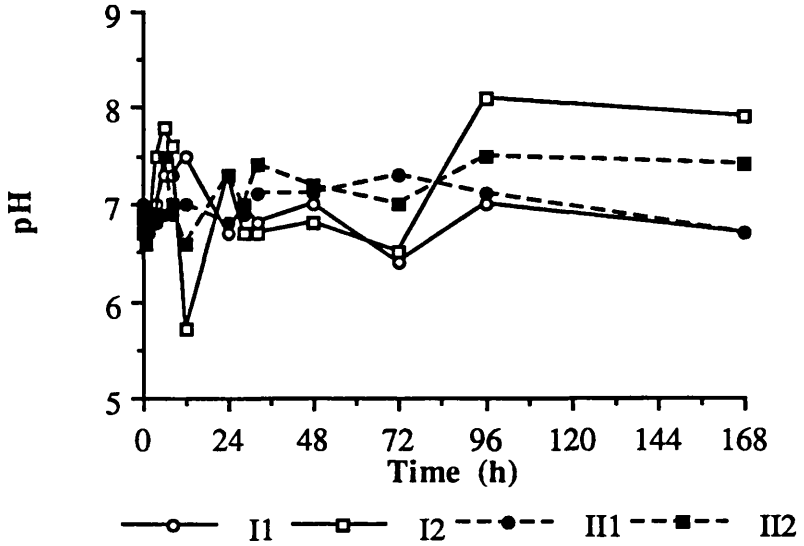

Figure 4-26. Caecal liquor pH following oral administration of ampicillin to ponies

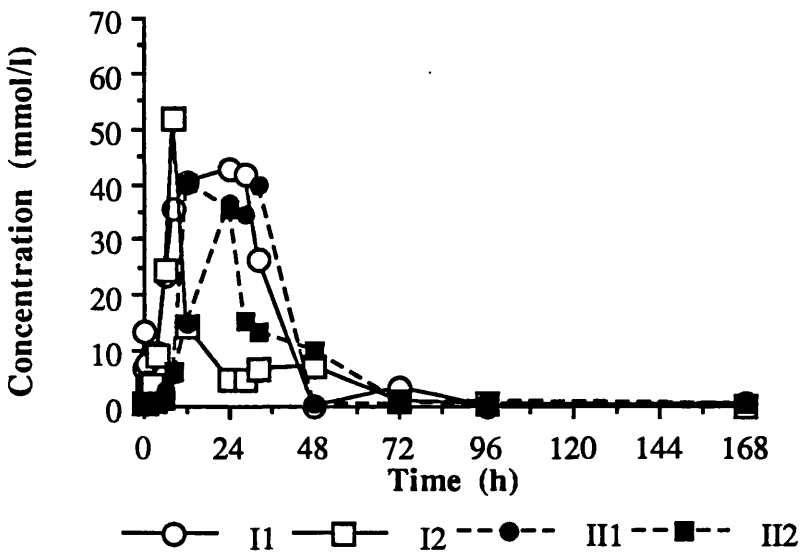

Figure 4-27. Lactic acid concentrations in caecal liquor following oral administration of ampicillin to ponies

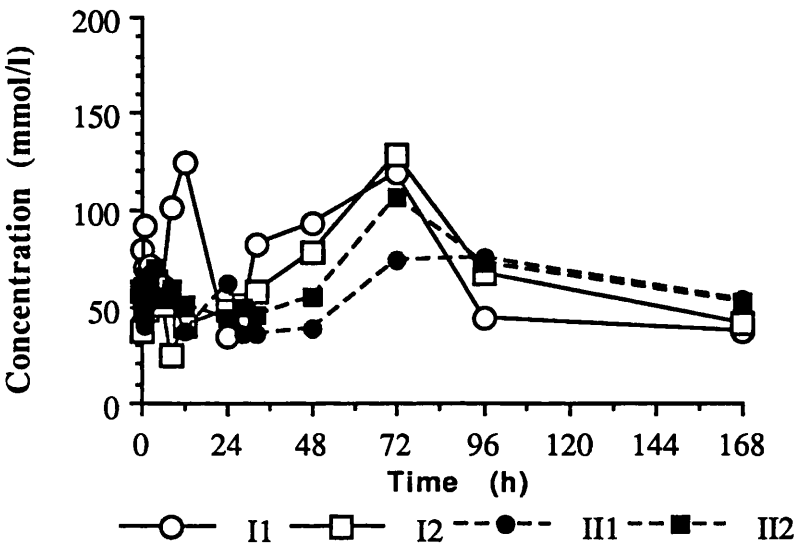

Figure 4-28. Total VFA concentrations in caecal liquor following oral administration of ampicillin to ponies

There were no marked alterations in total VFA concentrations in caecal liquor following oral administration of ampicillin (Figure 4-28). In pony I1, the total VFA concentration of 118.8 mmol/l at 12 h after drug administration was above the normal range of 24.4-109.2 mmol/l. There were variations in the individual acid concentrations that were outside the normal ranges. There was a reduction, compared with the normal range of 12.6-64.5 mmol/l, in the acetic acid concentrations to 4.4-10.6 mmol/l at 6, 12 and 24 h in pony I1 and at 8 and 12 h in pony I2. In pony I1, there was an increase in the concentrations of propionic acid to 41.5-99.9 mmol/l at 8, 12 and 72 h, compared with the normal range of 4.7-24.5 mmol/l. There were reductions in the concentrations of propionic acid to 0.0-3.8 mmol/l, compared with the normal range, at 24 h in pony I1, at 12 and 24 h in pony I2, at 6, 8, 12, 24, 28 and 32 h in pony II1, and at 8, 12, 24, 28 and 32 h in pony II2. The butyric acid concentrations of 1.2-2.7 mmol/l were lower than the normal range of 4.8-67.3 mmol/l at 8 h in pony I2 and at 48 h in pony II1.

The proportions of acetic, propionic and butyric acids, as a percentage of the total VFA concentrations, are given in Appendix B (Tables B44b, B45b, B46b and B47b). In pony I1, the acetic acid concentration ratio of 3.7-20.2%, at 4, 6, 12 and 24 h, was lower than the normal range of 27.9-67.0%. The ratio of propionic acid was increased to 41.2-84.1% at 6, 8 and 12 h in pony I1 and at 8 h in pony I2, compared with the normal range of 4.9-38.5%. The proportions of propionic acid were reduced to 0.0-3.8% at 24 h in pony I1, 12 and 24 h in pony I2, 8, 12, 24 and 28 h in pony II1 and at 12, 24 and 28 h in pony II2, compared with the normal range of 4.9-38.5%. In pony I1, the ratio of butyric acid was increased to 79.8% at 24 h, compared with the normal range of 8.7-66.7%, whereas in ponies I2 and II1 the ratio of butyric acid was reduced to 4.6-6.9% at 8 and 48 h, respectively. In pony I1, the alterations in acetic, propionic and butyric acid resulted in an increase in the ratio of propionic plus butyric acids to 76.9-93.9% (normal 27.1-72.1%) at 4, 6, 12, 24, and 48 h after drug administration.

Faecal SCFA concentrations are shown in Figures 4-29 and 4-30, and the individual data is given in Appendix B (Tables B48-B51). Faecal lactic acid concentrations were elevated to 35.6-64.2 mmol/kg at 24 and 48 h in pony I1, at 24, 48, 72 and 96 h in pony II1, and at 24, 48 and 96 h in pony II2 (Figure 4-29). The lactic acid concentrations were outside the normal range of 0.0-24.4 mmol/l at 24 h in pony I1, and at 24 and 48 h in ponies II1 and II2. There were considerable variations in individual VFA concentrations however none of these were related to the oral administration of penicillin G. The faecal total VFA concentrations were higher than normal at 168 h in pony I1, prior to drug administration to pony I2, and at 24 and 72 h in pony II2 (Figure 4-30). Faecal total VFA concentrations were lower than normal at 72 h in pony I2 and at 0 h in pony II1.

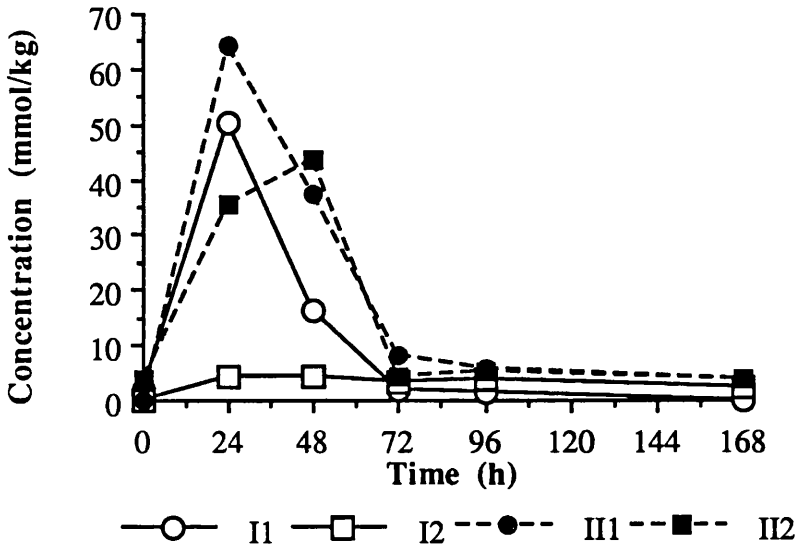

Figure 4-29 Lactic acid concentrations in faeces following oral administration of ampicillin to ponies

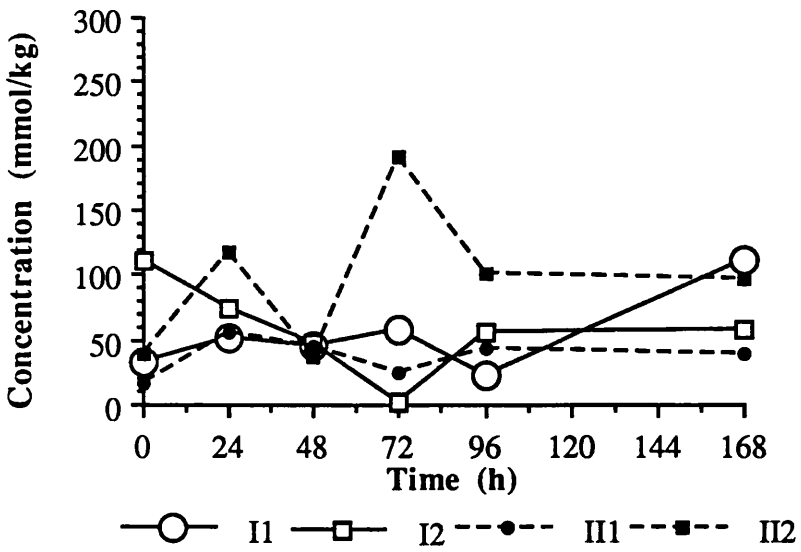

Figure 4-30. Total VFA concentrations in faeces following oral administration of ampicillin to ponies

4.5.5 Faecal dry matter content and consistency

The faecal dry matter content following oral administration of ampicillin is shown in Figure 4-31, and the individual data is given in Appendix B (Table B52). There were no marked alterations in faecal dry matter content. Although, the faecal dry matter content was slightly low (<17%) at 48 h in pony II1. No alterations in faecal consistency were observed.

4.6 Results of *in vitro* studies with ampicillin

4.6.1 Caecal liquor concentrations

A plot of caecal liquor concentrations (mean±SEM) following incubation *in vitro* is shown in Figure 4-32, and the individual and mean data are given in Appendix B (Tables B53a and b). After 3 h incubation there was an average of 16% of ampicillin remaining at the initial concentration of 0.25 μg/ml and 95% of ampicillin remaining in the 1, 5, 10, 20, 40 and 80 μg/ml samples. The largest reductions in concentration (zone diameter) were seen at either end of the concentration range due to limitations of the assay technique (zone diameter, limit of detection). Following 24 h incubation, there was an average of 36.4 % (excluding 0.25 and 1 μg/ml samples) of the drug activity remaining. There were no inhibition zones for the 0.25 μg/ml or 1 μg/ml sample following 24 h incubation.

4.6.2 SCFA concentrations

Lactic acid and total VFA concentrations (mean±SEM) following *in vitro* incubation of ampicillin in caecal liquor are shown in Figure 4-33, and the individual data is given in Appendix B (Tables B54-B58). There were no alterations in mean lactic acid or mean total VFA concentrations with time or drug concentration.

4.6.3 Acid pH

Ampicillin concentrations (mean±SEM) remaining following *in vitro* incubation at pH 1.9 for 1 h are shown in Figure 4-34, and the individual data is given in Appendix B (Table B59). There was little or no ampicillin destroyed by incubation at acid pH and on average there was 98.8% of the drug activity remaining.

4.6.4 Binding to hay

A plot of ampicillin concentrations (mean±SEM) remaining following *in vitro* incubation for 1 h with chopped hay versus initial concentrations is shown in Figure 4-35, and the results of the individual incubation experiments are given in Appendix B (Tables B60a and b). At pH 1.9, 64.6, 101.48, 116.62 and 106.62% remained, whereas at pH 7.0, an average of

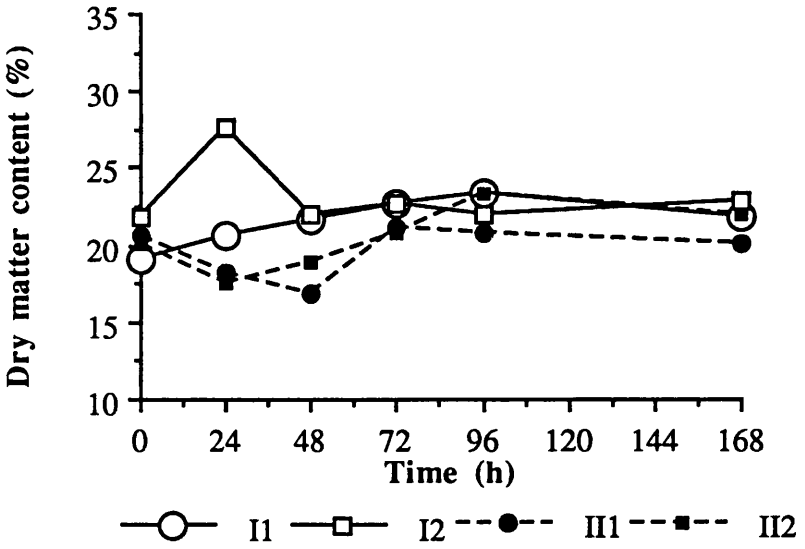

Figure 4-31. Faecal dry matter content following oral administration of ampicillin to ponies

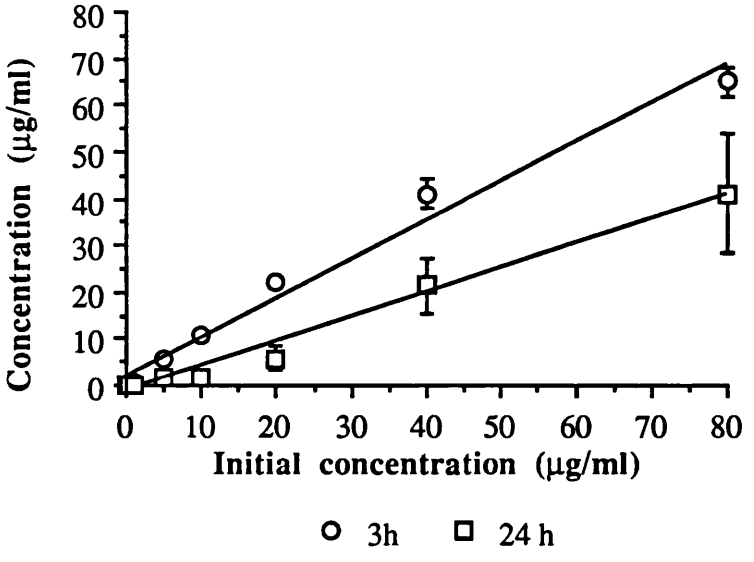

Figure 4-32. Concentrations (mean±SEM) of ampicillin in caecal liquor following incubation *in vitro* for 3 and 24 h

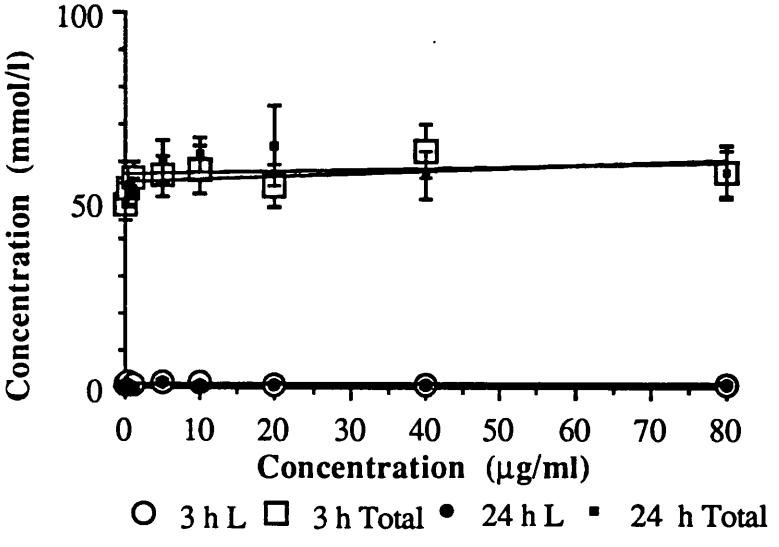

Figure 4-33. SCFA concentrations (mean±SEM) in caecal liquor following incubation *in vitro* with ampicillin for 3 and 24 h

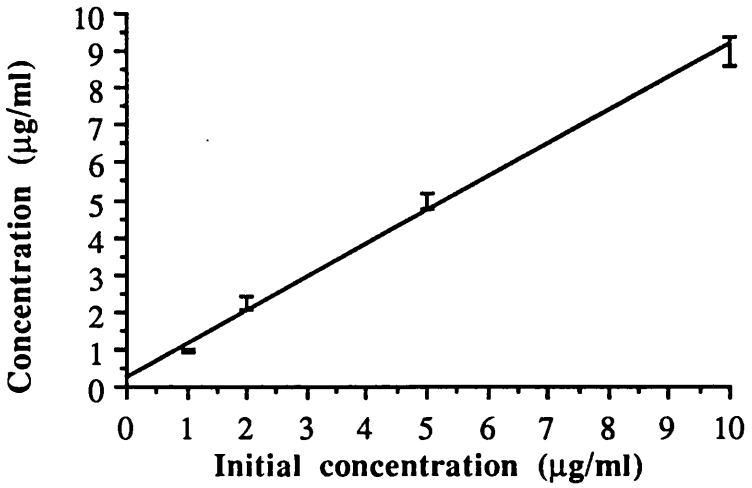

Figure 4-34. Concentrations (mean±SEM) of ampicillin following incubation of ampicillin at pH 1.9 for 1 h

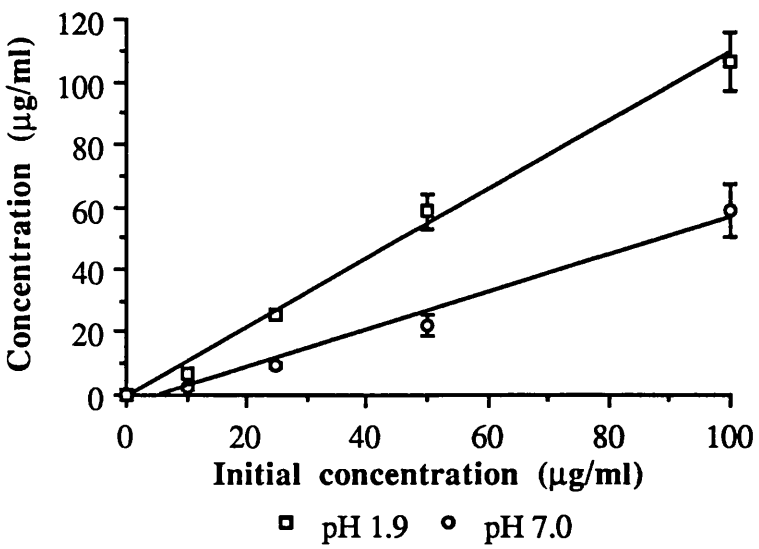

Figure 4-43. Concentrations (mean±SEM) of ampicillin following incubation *in vitro* with hay at pH 1.9 and pH 7.0 for 3 h

24.2, 36.0, 44.26 and 58.7% remained of the initial concentrations of 10, 25, 50 and 100 μg/ml. The extremely high percentages recorded at pH 1.9 were probably due to the pH of the solution effecting the rate of diffusion of the drug in the agar during the assay. However, it is interesting that 97.3% was free at pH 1.9, whilst only 40.8% of drug was measured at pH 7.0.

4.7 Discussion

The elimination half-life of ampicillin in horses, ponies and donkeys was similar to the elimination half-life in pigs (Galtier and Charpenteau, 1979) and calves (Black, 1976, Long *et al.*, 1983, Ziv and Horsey, 1979). The elimination half-life of ampicillin in horses and ponies was longer than in donkeys in the present study, but there were no statistically significant differences between the three groups. Baggot and Prescott (1987) stated that the elimination half-lives of the penicillins are short (0.5-1 h) and this was confirmed by the results of the present study (43-61 min). However, Dürr (1976) calculated an elimination half-life of ampicillin sodium of 93 min in the horse. In the present study, *Bacillus subtilis* was used as the test organism with a limit of detection of 0.02 μg/ml, whereas Dürr (1976) used *Sarcina lutea* and achieved a limit of detection of 0.05 μg/ml and administered ampicillin at a higher dose rate (40 mg/kg bwt). The slightly lower limit of detection in the present study should have lengthened rather than shortened the apparent elimination half-life. However, it may merely mean that small zones of inhibition were better defined in the present study. The range of the elimination half-lives reported in the present study was wide (34.08-148.74 min, 33.48-122.28 min and 30.06-66.12 min in horses, ponies and donkeys, respectively). The differences in elimination half-life may represent normal individual variations. Hence the importance of including large numbers of animals in studies of drug disposition and pharmacokinetics. The elimination half-life of ampicillin in the present study was similar to that reported by Dürr (1976) for penicillin G (53 min).

It is interesting that the mean AUC in horses was greater than in donkeys, and that there was a statistically significant difference ($p<0.05$) between the median AUC of 42.56 and 25.81 μg.h/ml in horses and donkeys, respectively. Similarly, the mean CL_b was slower in horses than in donkeys, and there was a statistically significant difference ($p<0.05$) between the median CL_b of 234.90 and 387.50 ml/h.kg in horses and donkeys, respectively. A similar difference was demonstrated by Kinabo and Bogan (1989) following administration of the flukicidal drug triclabendazole to horses, ponies and donkeys. It may be that horses have a lower metabolic capacity for some drugs than donkeys, however, ampicillin is not thought to be extensively metabolized prior to excretion. The differences observed in the present study may reflect a different capacity for renal excretion. The renal excretion of the ß-lactam antimicrobial agents is governed partly by an active transport system, and this may be

accelerated by increasing urinary pH. However, acidification and alkalisation of urine in the horse did not effect the pharmacokinetic variables calculated following intravenous administration of ampicillin sodium (Sarasola *et al.*, 1992). In addition, the effects of probenecid, a competitor of the active transport mechanism would be of use potentially in slowing the elimination of ampicillin from the body. In man, Arvidsson *et al.* (1981) showed that probenecid slowed the renal elimination of the cephalosporin, cefoxitin, after single and repeated doses. In the pig, Galtier and Alvinerie (1979) demonstrated that the systemic availability of ampicillin was enhanced following administration of probenecid due to a reduction in the plasma clearance of the former. In the horse, Juzwiak *et al.* (1989) reported a 50% reduction in the clearance of cephapirin (at a dose rate of 20 mg/kg bwt) in mares following concurrent administration of probenecid (at a dose rate of 50 mg/kg bwt) and Sarasola and McKellar (1992) reported a similar reduction in the CL_b of ampicillin in horses, following intravenous administration at a dose rate of 10 mg/kg bwt, due to the concurrent administration of probenecid (at a dose rate of 75 mg/kg bwt).

The overall effectiveness of penicillin therapy is influenced by the height of peak serum concentrations and the time, which is not necessarily continuous, that the effective serum concentrations are maintained. The cumulative area under the plasma concentration versus time curve determines the amount of antibiotic reaching the site of infection (Baggot, 1982) and the height of the peak concentration in serum determines the rate of penetration and concentration attained at the site of infection (Bergan, 1978). Powers *et al.* (1984) stated that tissue or blood concentrations that are greater than or equal to the MIC should be maintained throughout the period of therapy especially with the so-called bacteriostatic antimicrobial agents. Although the maintenance of plasma concentrations greater than the MIC is important for the bacteriostatic antimicrobial agents, it is less important for the bactericidal antimicrobial agents where intermittent exposure may be equally effective since bactericidal antimicrobial agents are active mainly against multiplying bacteria (Knifton, 1982). The peak serum concentration of a given antimicrobial agent must exceed a certain minimum concentration, which is some multiple of the MIC of a pathogen, such as four to eight times the MIC (Koritz 1984, Sande and Mandell, 1985). Intermittent or pulse dosing is not any more or less effective than continuous infusion. In the present study, plasma concentrations of greater than eight times the MIC of sensitive equine bacteria were achieved.

The dosage regimen necessary to maintain therapeutic plasma concentrations is disputed. Prescott and Baggot (1988a) and Van Miert (1988) recommend that a dose rate of 10-20 mg/kg bwt administered every 6-8 h was suitable. The dosage regimen suggested by Bywater (1982a) was 2-7 mg/kg bwt every 12-24 h. On the other hand, Huber (1982a) reported that currently the recommended dose rate for *Equidae* is 11-16 mg/kg bwt every 4-8 hours. It is difficult to predict the dosage interval precisely since some studies have indicated

hours. It is difficult to predict the dosage interval precisely since some studies have indicated that intermittent dosing with ß-lactam antimicrobial agents may be more efficient (Tauber *et al.*, 1984), whilst other studies have suggested that constant activity may be superior (Gerber *et al.*, 1983), and the choice may remain essentially empirical (Weinstein, 1977). The MIC of ampicillin for *Streptococcus equi* was 0.025-0.05 µg/ml (Keefe *et al.*, 1980), whereas the MIC of *Rhodococcus equi* was 4 µg/ml (Prescott and Nicholson, 1984). Zanon (1977) reported that the minimum antibiotic concentration of ampicillin was $^1/_8$-$^1/_{64}$ of the minimum bactericidal concentration. If a maintenance concentration of >0.1 µg/ml (*i. e.* four times 0.025 µg/ml) is desired then intravenous administration of ampicillin sodium, at a dose rate of 10 mg/kg bwt, would have to be repeated every 8 h (three times per day) in horses and every 6 h (four times per day) in ponies and donkeys. This is similar to the regimen recommended by Prescott and Baggot (1988a) and Van Miert (1988) of 10-20 mg/kg bwt every 6-8 h and would seem to be a sensible compromise, however less frequent dosing may be effective clinically.

In man, there is considerable variation (20-75%) in the systemic availability of ampicillin following oral administration (Jusko, 1975). Absorption of ampicillin was rapid following oral administration to ponies. However, the systemic availability of ampicillin was low (5.54, 3.37, 3.74 and 1.20% in ponies I1, I2, II1 and II2 respectively). Brown *et al.* (1984b) did not calculate the systemic availability of ampicillin trihydrate following oral administration to foals. However, the mean peak serum concentrations (5 µg/ml at 1 h) reported by Brown *et al.* (1984b) were higher than the peak plasma concentrations of ampicillin sodium reported in the present study (2.30, 1.16, 1.96 and 0.74 µg/ml at 0.5, 0.5, 0.75 and 0.5 h in ponies I1, I2, II1 and II2, respectively). This may be due to the fact that Brown *et al.* (1984b) administered ampicillin trihydrate at the higher dose rate of 20 mg/kg bwt. In the present study, the sodium salt of ampicillin was administered, whereas Brown *et al.* (1984b) administered the trihydrate salt. Different salts, and even different formulations, of a drug may have different systemic availabilities. However, it has been suggested that the sodium salt of ampicillin may be more effective clinically, compared with the trihydrate salt, through the production of higher concentrations in the body. It would be interesting to examine the effect of dose rate on the systemic availability of ampicillin sodium.

In man, amoxicillin is absorbed rapidly from the gastrointestinal tract following oral administration and has a systemic availability of 88.7% (70.9-105.5%) (Zarowny *et al.*, 1974). In the adult horse, the bioavailability of amoxicillin following oral administration was reported as 5.3% (Ensink *et al.*, 1992) and 10.4% (Wilson *et al.*, 1988). However, the systemic availability of amoxicillin (36.2±13.1% (mean±SD) at a dose rate of 20 mg/kg bwt and 42.7±15.0% at a dose rate of 30 mg/kg bwt) following oral administration to neonatal

foals was higher than in the adult horse (Baggot *et al.*, 1988). The peak concentrations of amoxicillin (2.03 μg/ml at 1 h) following oral administration at a dose rate of 15 mg/kg bwt were similar (Ensink *et al.*, 1992) to the peak plasma ampicillin concentrations described in the present study. In the present study, ampicillin was measured in plasma >0.1 μg/ml for 1.5-2 h following oral administration, thus dosing would require to be repeated frequently to maintain therapeutic plasma concentrations.

Ampicillin was measured in the caecal liquor following intravenous and oral administration to ponies. In the present study, an estimated 3.66% and 3.18% of the administered ampicillin were measured in caecal liquor from 1 and 0.25 h after intravenous administration to pony I on occasions 1 and 2, respectively. Following intravenous administration of ampicillin to pigs, around 1% was eliminated in bile within 4 h (Galtier and Alvinerie, 1979), and ampicillin was measured in caecal contents from 30 min onwards (Galtier and Charpenteau, 1979). In the present study, there was considerable variation in peak caecal liquor concentrations following oral administration (101.31, 144.53, 47.40 and 3.63 μg/ml in ponies I1, I2, II1 and II2, respectively). In the calf, amoxicillin was measured in caecal contents from 0.5 h, after oral administration at a dose rate of 7 mg/kg bwt, at concentrations (mean±SEM) of up to 635.5±79.5 μg/g at 6 h (Palmer *et al.*, 1977). The inter-animal variation in peak caecal liquor concentrations in the present study was possibly due to the difference between the two animals in the stomach to caecum transit time; the peak concentrations were reached at 2 h after drug administration to ponies I1 and I2, and at 6 h after drug administration to ponies II1 and II2. Moreover, the length of time that ampicillin remained in the caecum (MRT) was shorter in ponies I1 and I2 than in ponies II1 and II2 (3.92, 1.98, 6.75 and 6.27 h, respectively). Although, there were differences in the peak caecal liquor concentrations, the AUC of ampicillin in the caecal liquor was of a similar magnitude (450.82, 188.18 and 273.81 μg.h/ml, respectively) in ponies I1, I2 and II1. However, in pony II2 the peak caecal liquor concentration was 3.63 μg/ml only, and the AUC of ampicillin reaching the caecal liquor was 8.37 μg.h/ml. This may have been due to the destruction of ampicillin in the gastrointestinal lumen by, *e. g.* ß-lactamase enzymes, due to differences in the gastrointestinal microflora between animals and even between occasions.

There was no ampicillin detected in faecal samples following intravenous or oral administration. It seems unlikely that the high concentrations of ampicillin in caecal liquor were absorbed in the caecum or colon since ampicillin was detected in plasma for up to 4 h only, although plasma samples were taken for up to 48 h following oral administration. Incubation of ampicillin *in vitro* for 24 h resulted in destruction of around 70% of the initial concentrations of 0.25-80 μg/ml. There may have been destruction of ampicillin in the colon or in the faecal samples, *e. g.* by ß-lactamase enzymes, prior to analysis. However, faecal

samples were stored at 4 °C, alongside caecal liquor samples, prior to analysis and high concentrations were present in caecal liquor following storage.

There was an apparent increase in the number of viable coliforms, streptococci, lactobacilli and *Clostridium spp.* isolated from caecal liquor following oral administration of ampicillin. The alterations in the number of coliforms, streptococci, lactobacilli and *Clostridium spp.* reported in the present study were not dissimilar to those recorded by Andersson *et al.* (1971) and White and Prior (1982) following intravenous and oral administration, respectively, of oxytetracycline. There were no marked alterations in the caecal liquor pH following intravenous administration of ampicillin. However, there were both increases and decreases in caecal liquor pH following oral administration of ampicillin. This result is not surprising since there were alterations in the SCFA concentrations. The most marked alterations in the SCFA concentrations were in the caecal liquor lactic acid concentrations. There were increases in the caecal liquor lactic acid concentrations in one pony on both occasions following intravenous administration of ampicillin, but these were much more pronounced and occurred in both ponies following oral administration. In addition, faecal lactic acid concentrations were elevated in three animals following oral drug administration. Thus, it appears that high caecal liquor lactic acid concentrations are reflected by elevated faecal lactic acid concentrations and suggests that lactic acid, at least at high concentrations, was not absorbed in the large intestine. The increase in the lactic acid concentrations could be explained by the increase in the number of lactic acid producing bacteria such as streptococci, lactobacilli and *Clostridium spp.* isolated. The peak in lactic acid concentrations may have been due to an increase in the production of D- or L-lactic acid. The increased lactic acid concentrations in the present study occurred at variable times following intravenous and oral administration of ampicillin although they always occurred following the peak drug concentration. Interestingly, there was an increase in the caecal liquor lactic acid concentrations in the only pony in which drug concentrations were measured in caecal liquor following intravenous administration of ampicillin.

There were changes in the concentrations of individual VFAs following both intravenous and oral administration of ampicillin. There were alterations in the VFA concentrations in pony I following intravenous administration of ampicillin.There were no alterations in the caecal liquor VFA concentrations where no ampicillin was detected in the caecal liquor. Following oral administration of ampicillin, the most obvious trend was a reduction in the propionic acid concentrations in caecal liquor. In addition, there were both increases in the acetic acid concentrations, increases in the concentrations and proportions of propionic acid, and an apparent reduction in the concentrations and proportions of butyric acid following oral administration of ampicillin. It is interesting to note that the peak lactic acid concentrations almost always coincided with the lowest propionic acid concentrations in

caecal liquor. Moreover, the alterations in the propionic acid concentrations in caecal liquor were similar in both ponies on both occasions despite the differences in peak caecal liquor concentration. In addition, the reduction in the caecal liquor propionic acid concentrations was shorter in ponies I1 and I2 (up to 12 h) than in ponies II1 and II2 (up to 32 h). Although, it would have been useful to have sampled between 12 and 24 h after drug administration in pony II in particular. The peak caecal liquor concentrations were higher in ponies I1 and I2, but the MRT was longer in ponies II1 and II2. This suggests that the alterations in propionic acid concentrations may be related to the length of time that the drug was present in the caecum and not to peak concentrations. Thus, it may be that ampicillin can alter bacterial metabolism in the caecum even if it is destroyed rapidly. A shift in fermentation away from acetate, butyrate and methane production towards propionate production is the basis of the use of antimicrobial agents for growth promotion in ruminants and is associated with defaunation (Demeyer and Van Nevel, 1987, Prins, 1987). A prolonged reduction in propionic acid concentrations in caecal liquor may represent a reduction in VFA production and may result in a reduction in glucose available to the animal since propionate *via* succinate is the main source of glucose in the animal.

It is interesting to note that a high percentage (*circa* 59%) of ampicillin was bound to hay following *in vitro* incubation at pH 7.0. This would mean that only 40% would be available for absorption in the small intestine. However, at acid pH, similar to that seen in the stomach, essentially no drug was bound to hay (*circa* 3%). Ziv *et al.* (1977) noted that fasting of calves improved the systemic availability of ampicillin by 2.67 times. In the adult horse, pivampicillin had a bioavailability of 30.9% in starved horses and 35.9% in fed horses (Ensink *et al.*, 1992). The withholding of food prior to drug administration may improve absorption. However, this would be difficult to maintain for prolonged periods in a clinical situation. Moreover, unless there was a substantial improvement in the systemic availability by withholding food prior to oral administration of ampicillin sodium in the equine, the risk of inducing antimicrobial-associated diarrhoea would be high due to the high concentrations of ampicillin reaching the large intestine.

Intravenous administration of ampicillin sodium at a dose rate of 10 mg/kg bwt every 6-8 h would provide and maintain suitable plasma concentrations for the treatment of infections caused by susceptible bacteria in horses, ponies and donkeys. Oral administration at a dose rate of 10 mg/kg bwt would be impractical for the treatment of systemic bacterial infections in the equine. An increase in the oral dose rate of ampicillin would probably produce higher plasma concentrations. However, it is contraindicated because it would result in very high intestinal drug concentrations and may result in the development of enterocolitis.

5 Studies with amikacin

5.1 Introduction

The aminoglycoside antimicrobial agents are large polar organic bases with a low lipid solubility, poor membrane penetration and increased activity at alkaline pH. The aminoglycosides are absorbed poorly from the gastrointestinal tract but retain their activity within it (English and Roberts, 1979). They are bactericidal antimicrobial agents which act on ribosomal protein synthesis. Aminoglycosides containing 2-deoxystreptamine (kanamycin, gentamicin, tobramycin, netilmicin and amikacin) are valuable for the treatment of infections caused by Gram negative bacteria (Price and Siskin, 1984). Resistance to these agents is due to a plasmid-mediated modification of the antimicrobial agent by a bacterial enzyme and, less commonly, to a defect in the transfer of the antimicrobial agent to the intracellular ribosomal target site in susceptible bacteria (Price and Siskin, 1984). However, the use of the aminoglycosides is limited by their low therapeutic index (Prescott and Baggot, 1985). The adverse side effects that occur following administration include ototoxicity and nephrotoxicity.

A variety of aminoglycosides, including streptomycin, neomycin, kanamycin, gentamicin and amikacin, are used in veterinary practice. In the equine, a wide range of dose rates have been suggested for their administration. Baggot *et al.* (1981) suggested that intravenous or intramuscular administration of streptomycin at a dose rate of 10 mg/kg bwt every 12 h would be suitable. In their review Roberts and English (1979) suggested that intravenous or oral administration of neomycin at a dose rate of 20-50 mg/kg bwt every 6-8 h was a suitable dosage regime. However, Baggot *et al.* (1981) and Baggot *et al.* (1985) noted that intravenous or intramuscular administration of neomycin at a dose rate of 10 mg/kg bwt every 12 h would provide therapeutic plasma concentrations and avoid the development of signs of toxicity. Boyd and Allen (1988) reported the use of neomycin at a dose rate of 3 mg/kg bwt as an intrauterine infusion. Knight (1975) recommended the administration of kanamycin at a dose rate of 5 mg/kg bwt every 8 h, however Baggot *et al.* (1981) suggested that intravenous or intramuscular administration of kanamycin at a dose rate of 10 mg/kg bwt every 8 h would be appropriate, and Roberts and English (1979) recommended the dose rates of 11-15 mg/kg bwt every 8-12 h. Dose rates ranging from 2 to 18 mg/kg bwt every 6-12 h have been recommended for intravenous or intramuscular administration of gentamicin (Baggot *et al.*, 1986, English and Roberts, 1979, Gilman *et al.*, 1987, Haddad *et al.*, 1985, Pedersoli *et al.*, 1980, Riviere *et al.*, 1983a, Smith *et al.*, 1988).

Amikacin is produced by the chemical alteration of kanamycin A (Monteleone *et al.*, 1983). Frequently, it has the advantage of being effective in the treatment of infections due to

organisms resistant to other aminoglycosides (Cudd, 1985, Gingerich *et al.*, 1983). Amikacin retains its antimicrobial activity in the the presence of around 90% of the different enzymes which destroy other aminoglycoside antimicrobial agents, and it has potent activity against *Pseudomonas spp.*, *E. coli*, *Proteus spp.*, *Providencia spp.*, *Klebsiella-Enterobacter-Serratia spp.*, *Acinebacter spp.*, *Citrobacter freundii*, and non-ß-lactamase and ß-lactamase producing staphylococci (Caudle *et al.*, 1983, Gingerich *et al.*, 1983). In addition, resistance to amikacin is slow to develop (Price and Siskin, 1984) and it has been shown that resistance to gentamicin and tobramycin was reduced when amikacin was used as the aminoglycoside of choice (Gingerich *et al.*, 1983). However, Price and Siskin (1984) reported that there were no alterations in resistance patterns due to amikacin usage. The amikacin preparation which is available at present, in veterinary medicine, was designed for use as an intrauterine infusion in mares. Brook (1982) suggested that an infusion of 2 g of amikacin dissolved in 250 ml of normal saline daily for 5 days was a suitable regime for treating susceptible bacterial infections of the genital tract in mares. In the equine, intravenous or intramuscular administration of amikacin at dose rates ranging from 4.4 to 7.0 mg/kg bwt every 12 h have been recommended (Adland-Davenport *et al.*, 1990, Brown *et al.*, 1984c, Brown *et al.*, 1986, Orsini *et al.*, 1985).

Aminoglycosides are retained within the gastrointestinal lumen following oral administration. In man, this property has been utilized to suppress the gastrointestinal flora prior to colon surgery (Gorbach, 1983). Neomycin is used commonly, in combination with the macrolide erythromycin, to produce a concentration of 1-3 mg/ml of neomycin in the colonic contents, which is suitable to suppress the gastrointestinal flora (Gorbach, 1983). However, it has been shown that a number of rumen bacteria are not inhibited by the presence of kanamycin, neomycin and streptomycin (Prins, 1987). In fact, at concentrations of 5, 10 and 20 µg/ml kanamycin, neomycin and streptomycin had no effect on cellulose breakdown in rumen fluid. However, ruminal function was depressed in sheep following oral administration of aminoglycoside antimicrobial agents at a dose rate of 0.5-1.0 g/day (Prins, 1987). In addition, amikacin is not absorbed beyond the endometrium following intrauterine administration to mares (Caudle *et al.*, 1983).

The purpose of the present study was to determine the plasma disposition and pharmacokinetics of amikacin sulphate following intravenous administration at a dose rate of 6 mg/kg bwt to horses, ponies and donkeys. Alterations in the intestinal flora were studied by bacteriological examination of serial faecal samples and large intestinal fermentation was studied using faecal SCFA concentrations. The faecal dry matter content and the appearance of faeces were used as indicators of the presence or absence of diarrhoea. Similar studies were carried out in ponies with cannulated caecal fistulas following a single intravenous or oral administration, *via* nasogastric tube, of amikacin sulphate at a dose rate of 6 mg/kg bwt.

In addition, drug, bacteriological and SCFA analyses were carried out on caecal liquor, and plasma biochemistry and haematology were monitored.

5.2 Materials and Methods

5.2.1 Intravenous administration of amikacin to horses, ponies and donkeys

Three Thoroughbred geldings (No. 3-5), four ponies (No. 7-10) and three donkeys (No. 14-16) were used as outlined in the general Materials and Methods. Amikacin sulphate was administered by intravenous bolus injection at a dose rate of 6 mg/kg bwt. Plasma samples were taken at 0, 0.033, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h. Faecal samples were taken at 0, 24 and 48 h for drug analysis, bacteriological examination, SCFA analysis and measurement of faecal dry matter content.

5.2.2 Intravenous administration of amikacin to ponies with cannulated caecal fistulas.

Two pony mares with cannulated caecal fistulas (I and II), as outlined in the general Materials and Methods, were used on up to 2 occasions (1 and 2). Amikacin sulphate was administered by intravenous bolus injection at a dose rate of 6 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis, measurement of pH and SCFA analysis were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, with additional plasma samples at 0.033 and 0.083 h and an additional caecal liquor sample at 48 h. Bacteriological examination of caecal liquor was carried out at 0, 24 and 48 h. Faecal samples were taken at 0, 24 and 48 h for drug analysis, SCFA analysis and the measurement of dry matter content. Plasma biochemistry and haematological examinations were carried out at 0, 24 and 48 h on occasion 1 and 0, 24 and 120 h on occasion 2.

5.2.3 Oral administration of amikacin to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 2 occasions (1 and 2) at least 5 days after intravenous administration of amikacin sulphate. Amikacin sulphate was administered *via* nasogastric tube at a dose rate of 6 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis, pH measurement and SCFA analysis were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, with further plasma samples at 0.033 and 0.083 h, and caecal liquor samples taken at 28, 32, 48, 52, 56, 72, 96 and 168 h. Bacteriological examinations of caecal liquor were carried out at 0, 24, 48, 72, 96 and 168 h. Faecal samples were taken for the measurement of drug concentrations, SCFA concentrations and dry matter content at 0, 24, 48, 72, 96 and 168 h. Samples were taken for plasma

biochemistry and for haematological examinations at 0, 24, 48 and 168 h, with an additional haematology sample at 72 h.

In addition, amikacin sulphate was administered by nasogastric tube to pony 13 on one occasion. Plasma samples were taken at 0 to 24 h with no samples at 0.033 and 0.083 h. Faecal samples were taken at 0, 24, 48 and 72 h for drug analysis, bacteriological examination, SCFA analysis and the measurement of dry matter content.

5.2.4 *In vitro* studies with amikacin

Amikacin sulphate was incubated with caecal liquor for 3 and 24 h and drug concentrations and SCFA concentrations were measured as described in the general Materials and Methods. In addition, amikacin concentrations were measured following incubation at pH 1.9 as outlined in the general Materials and Methods. Also, concentrations of amikacin were measured following incubation at pH 1.9 and 7.0 in the presence of chopped hay as outlined in the general Materials and Methods.

5.3 Results of intravenous administration of amikacin to horses, ponies and donkeys

5.3.1 Plasma disposition and pharmacokinetics

A semilogarithmic plot of plasma concentrations (mean±SEM) following intravenous administration of amikacin to horses, ponies and donkeys is shown in Figure 5-1. The plasma concentration versus time data is given in Appendix C (Tables C1-C3). In all 3 groups, the initial plasma concentration versus time plots were similar, however the decline phase of the plasma concentration versus time plot was similar in horses and donkeys and declined more steeply in ponies, suggesting that the elimination of amikacin was slower in horses and donkeys than in ponies. Amikacin was detected in plasma (>0.02 μg/ml) for up to 12 h in horses and donkeys and 8 h in ponies (there was no 12 h sample in ponies).

The pharmacokinetic parameters calculated from the bi-exponential equations used to describe the plasma concentration versus time data are given in Table 5-1, and the individual data is given in Appendix C (Tables C4-C6). There were no statistically significant differences between the pharmacokinetic parameters of horses, ponies and donkeys calculated using a Kruskal Wallis test. The harmonic mean of the elimination half-life calculated for horses, ponies and donkeys was similar (1.6-3 h). The elimination half-life was longest in horses (2.84 h) and shortest in ponies (1.60 h). Similarly, the MRT was longest in horses, followed by donkeys and then ponies. The AUC and AUMC were lowest in ponies with the calculated AUC around 50% of the horse value and the AUMC around

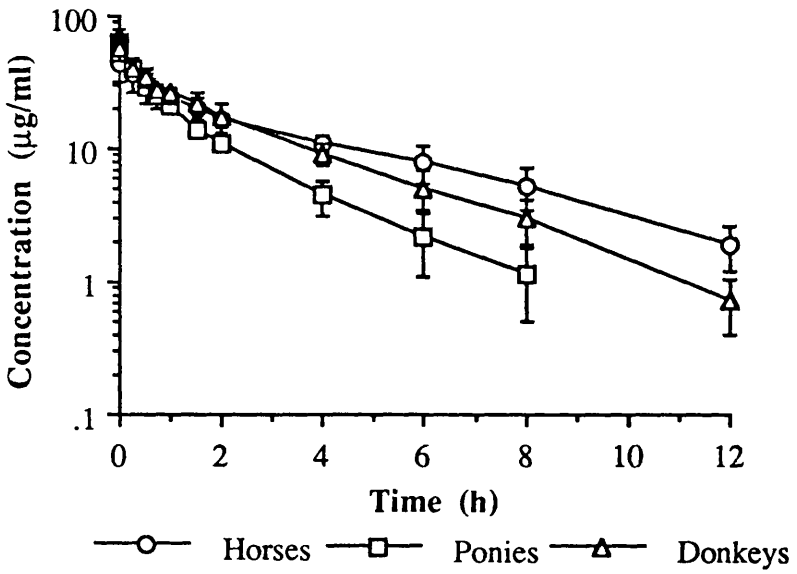

Figure 5-1 Plasma concentrations (mean±SEM) following intravenous administration of amikacin to horses, ponies and donkeys

Parameter	Horses (n=3)	Ponies (n=4)	Donkeys (n=3)
t1/2 B2 (h)*	0.10	0.20	0.05
t1/2 B1 (h)*	2.84	1.60	1.93
Cp0 (µg/ml)	64.92±18.37	64.77±2.82	87.97±11.45
Vc (ml/kg)	117.93±45.39	93.18±4.18	70.34±8.19
AUC_{obs} (µg.h/ml)	136.15±12.21	83.87±14.15	119.96±25.84
$AUMC_{obs}$ (µg.h^2/ml)	553.82±134.92	203.37±62.99	355.86±106.87
AUC (µg.h/ml)	134.69±11.41	77.61±11.84	116.26±24.81
AUMC (µg.h^2/ml)	639.83±191.93	173.84±55.49	340.87±103.70
MRT (h)*	4.01±0.86	2.28±0.29	2.82±0.35
Vd_{area} (ml/kg)	214.87±58.34	191.28±13.51	156.85±15.94
Vd_{ss} (ml/kg)	206.58±59.11	162.73±7.25	150.21±15.97
CL_b (ml/h.kg)	45.19±3.76	82.37±11.27	57.98±15.05
k_{el} (/h)	0.49±0.14	0.90±0.15	0.82±0.18
k_{21} (/h)	3.04±1.09	1.69±0.36	5.05±1.70
k_{12} (/h)	3.50±2.40	1.22±0.26	6.39±2.79

Table 5-1. Disposition kinetics of amikacin in plasma following intravenous administration to horses, ponies and donkeys

Key: data as mean±SEM; * harmonic mean

33.3% of the horse value. In addition, the mean CL_b and k_{el} in horses were around twice and one-half of the CL_b and k_{el} calculated in ponies, respectively. The other calculated parameters were similar in each of the 3 groups. The distribution rate constants (k_{21} and k_{12}) were similar in horses, ponies and donkeys, but the distribution rates were faster in horses and donkeys than in ponies.

The plasma disposition of amikacin was best described by a tri-exponential equation in horse 3, pony 10, donkey 14 and donkey 15 where there were 7, 4, 6 and 6 points making up the elimination phase, respectively. The pharmacokinetic parameters calculated from the tri-exponential equations used to describe the plasma concentration versus time data are given in Table 5-2. The movement of drug between the first and second compartments, (rate constants k_{21} and k_{12}) 2.99-17.57/h and 1.16-12.48/h, respectively, was faster than movement of drug between the first and third compartments, (rate constants k_{31} and k_{13}) 0.87-2.57/h and 0.22-3.44/h, respectively. In addition, k_{21} was generally faster than k_{12}, except in donkey 15 where k_{21} and k_{12} were similar, and k_{31} was faster than k_{13}, except in horse 3 where they were similar.

5.3.2 Faecal concentrations

No amikacin was detected in faeces following intravenous administration to horses, ponies and donkeys.

5.3.3 Bacteriological examinations

Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of amikacin are shown in Figures 5-2, 5-3 and 5-4, and the individual data is given in Appendix C (Tables C7-C9).

Salmonella spp. and *C. difficile* were selected for but not isolated.

There were no marked changes in the number of coliforms, streptococci, lactobacilli, *Bacteroides spp.* or *Clostridium spp.* isolated from horses following intravenous administration of amikacin (Figure 5-2). The mean number of streptococci was raised to 10^8-10^9/g prior to and 48 h after drug administration. There were high numbers of streptococci isolated from horse 3 (10^8-10^{10}/g) throughout the study, and from horse 5 (10^8/g) at 24 h after drug administration. There were low numbers of *Bacteroides spp.* (10^6-10^8/g) isolated from horse 3 at 48 h, from horse 4 throughout the study, and from horse 5 prior to and at 48 h after drug administration.

Parameter	Horse 3	Pony 10	Donkey 14	Donkey 15
t1/2 B3 (h)	0.02	0.13	0.14	0.03
t1/2 B2 (h)	0.16	0.51	0.58	0.24
t1/2 B1 (h)	3.30	1.50	2.31	1.49
Cp0 (μg/ml)	103.67	68.34	74.13	81.78
Vc (ml/kg)	57.88	87.80	80.94	73.37
AUC_{obs} (μg.h/ml)	160.05	70.46	131.17	70.70
$AUMC_{obs}$ (μg.h^2/ml)	687.96	130.76	409.29	149.91
AUC (μg.h/ml)	155.62	64.85	126.24	68.10
AUMC (μg.h^2/ml)	716.96	107.78	388.64	139.91
MRT (h)	4.30	1.86	3.12	2.12
Vd_{area} (ml/kg)	185.28	200.50	158.61	189.32
Vd_{ss} (ml/kg)	177.64	153.77	146.32	181.03
CL_b (ml/h.kg)	38.56	92.52	47.53	88.11
k_{el} (/h)	0.67	1.05	0.59	1.20
k_{21} (/h)	17.57	3.54	2.99	10.24
k_{12} (/h)	12.33	1.16	1.78	12.48
k_{31} (/h)	2.51	0.87	1.04	2.57
k_{13} (/h)	3.44	0.37	0.22	0.64

Table 5-2. Disposition kinetics of amikacin in plasma following intravenous administration to horse 3, pony 10, donkey 14 and donkey 15

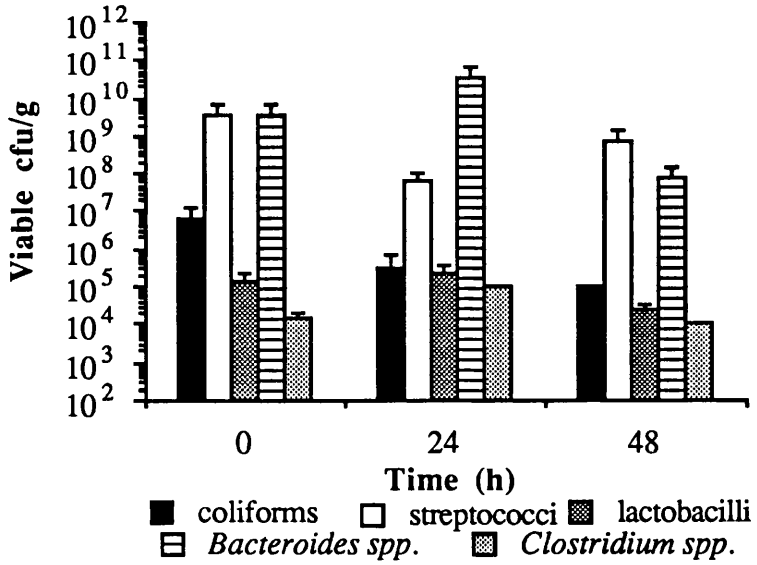

Figure 5-2. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of amikacin to horses

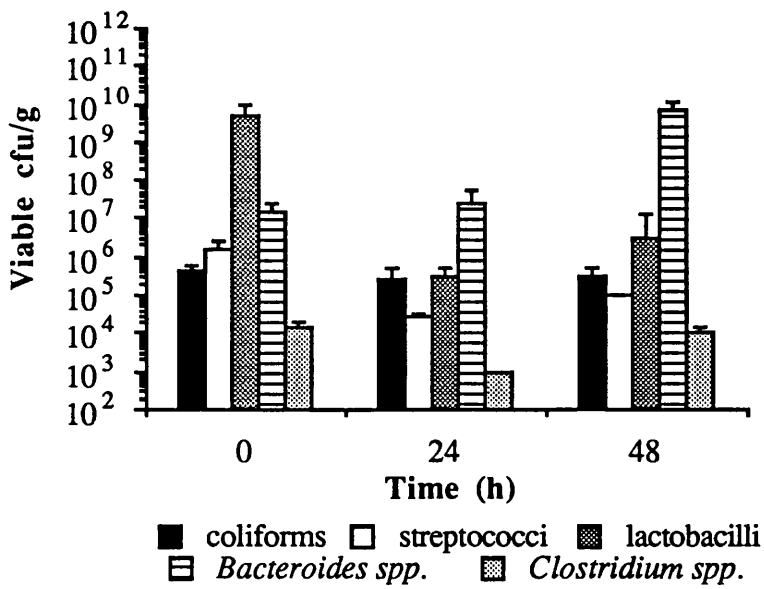

Figure 5-3. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of amikacin to ponies

There were no marked changes in the number of coliforms, streptococci, lactobacilli, *Bacteroides spp.* or *Clostridium spp.* isolated following intravenous administration of amikacin to ponies (Figure 5-3). The mean number of lactobacilli was high (10^9/g) prior to drug administration due to a high number of lactobacilli (10^{10}/g) isolated from pony 9. The mean number of *Bacteroides spp.* isolated was high at 48 h after drug administration. There were low numbers of *Bacteroides spp.* (10^5-10^8/g) isolated from all the ponies throughout the study, except from ponies 8 and 10 at 48 h when normal numbers (10^9-10^{10}/g) of *Bacteroides spp.* were isolated.

There were no marked changes in the numbers of coliforms, streptococci, lactobacilli, *Bacteroides spp.* or *Clostridium spp.* isolated from the 3 donkeys following the intravenous administration of amikacin (Figure 5-4). There were no streptococci isolated, on Slanetz and Bartley agar, from donkey 15 prior to the single intravenous administration of amikacin. The number of *Bacteroides spp.* isolated from donkeys faeces was low (10^5-10^7/g) on all occasions, except from donkey 15 (10^9/g) prior to drug administration. There were no *Clostridium spp.* isolated from donkeys 15 and 16. The number of *Clostridium spp.* isolated from donkey 14 prior to drug administration was quite high (10^7/g).

Bacteria isolated and identified using the API system were *Actinomyces israelii, Bifidobacter adolescentis* and *Eubacterium limosum.*

5.3.4 Faecal SCFA concentrations

Faecal lactic acid and total VFA concentrations (mean±SEM) following intravenous administration of amikacin to horses, ponies and donkeys are shown in Figures 5-5 and 5-6, and the data from individual animals is given in Appendix C (Tables C10-C20). The faecal SCFA concentrations were very variable following intravenous administration of amikacin to horses, ponies and donkeys. The faecal lactic acid concentrations were low following intravenous administration of amikacin to horses, ponies and donkeys, and were all within the normal range of 0.0-24.4 mmol/l. In horses and donkeys, the mean total VFA concentrations were within the normal range of 24.4-109.2 mmol/l at 0, 24 and 48 h, after intravenous administration of amikacin. In ponies, the mean total VFA concentrations of 0.0 mmol/l at 0, 24 and 48 h were lower than the normal range. There was considerable variation in total and individual VFA concentrations in individual animals however, these alterations were not considered to be associated with the intravenous administration of amikacin.

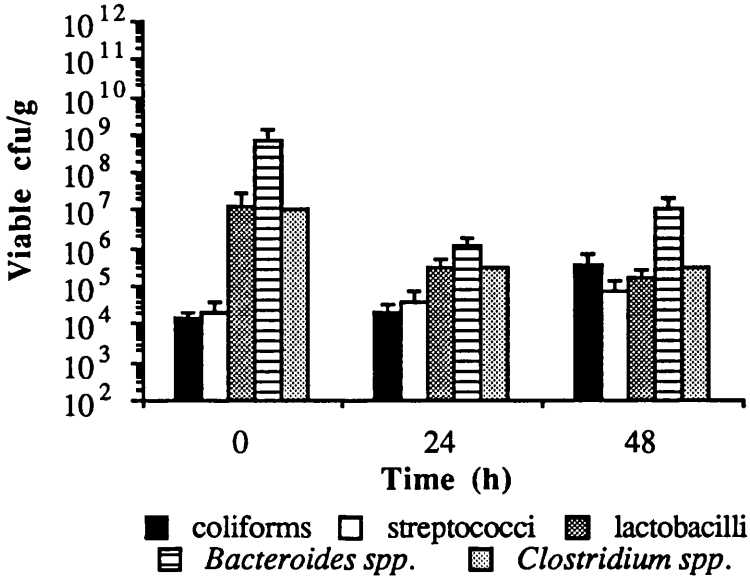

Figure 5-4. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of amikacin to donkeys

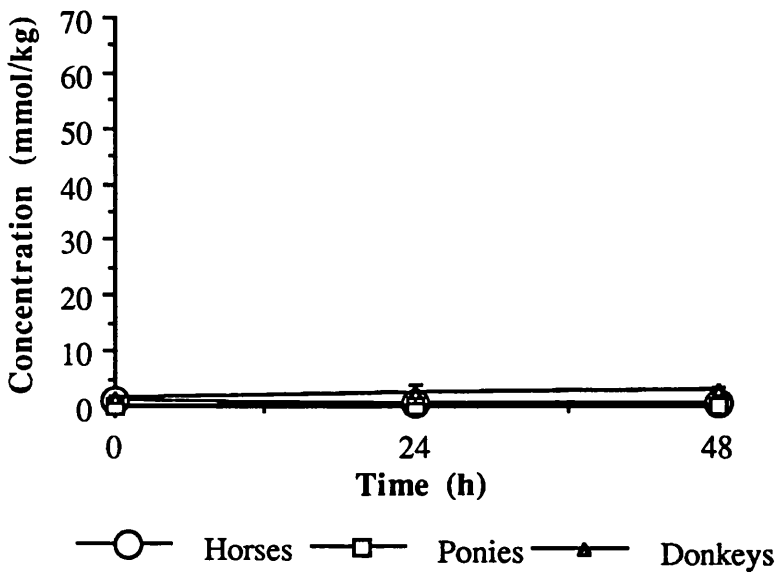

Figure 5-5. Lactic acid concentrations (mean±SEM) in faeces following intravenous administration of amikacin to horses, ponies and donkeys

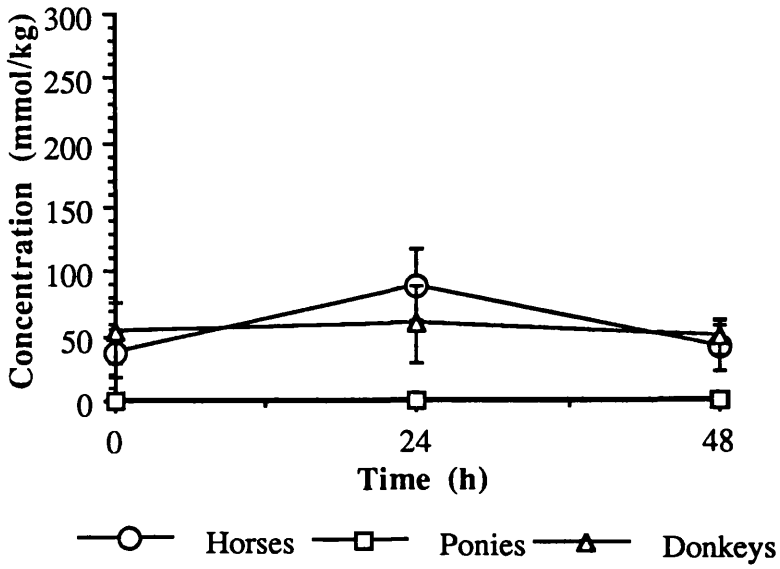

Figure 5-6. Total VFA concentrations (mean±SEM) in faeces following intravenous administration of amikacin to horses, ponies and donkeys

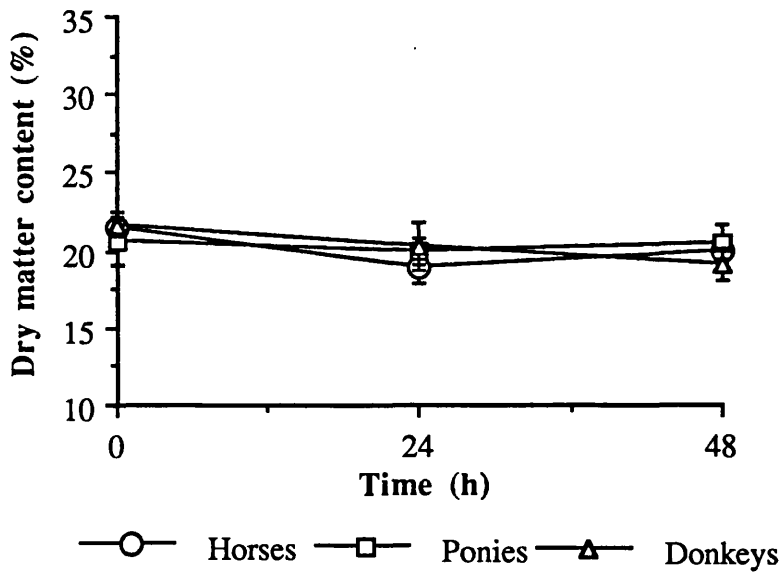

Figure 5-7. Faecal dry matter content (mean±SEM) following intravenous administration of amikacin to horses, ponies and donkeys

5.3.5 Faecal dry matter content and consistency

The faecal dry matter content (mean±SEM) following intravenous administration of amikacin to horses, ponies and donkeys is shown in Figure 5-7, and the data from individual animals is given in Appendix C (Tables C21-C23). There were no marked alterations in faecal dry matter content following intravenous administration of amikacin to horses, ponies and donkeys. The faecal dry matter content was slightly low (16.88%) in pony 7 prior to drug administration. There were no marked alterations of faecal consistency observed following intravenous administration of amikacin to horses, ponies and donkeys.

5.4 Results of intravenous administration of amikacin to ponies with cannulated caecal fistulas

5.4.1 Plasma disposition and pharmacokinetics

The plasma concentrations of amikacin following intravenous administration to ponies I and II on occasions 1 and 2 are given in Appendix C (Table C24).

A bi-exponential equation best described the data from ponies I1, I2 and II2. The pharmacokinetic parameters calculated from the plasma concentration versus time data of each animal on each occasion are given in Table 5-3. In addition, the pharmacokinetic parameters calculated using the tri-exponential equation which best described the plasma concentration versus time data from pony II1 are given in Table 5-3.

5.4.2 Caecal liquor and faecal concentrations

There was no amikacin detected in caecal liquor or faecal samples from ponies following intravenous administration.

5.4.3 Bacteriological examinations

Counts of viable bacteria in caecal liquor following intravenous administration of amikacin are shown in Figures 5-8 to 5-12, and the individual data is given in Appendix C (Table C25).

Salmonella spp. and *C. difficile* were selected for but not isolated.

There were no marked alterations in the number of coliforms (Figure 5-8), streptococci (Figure 5-9), lactobacilli (Figure 5-10), *Bacteroides spp.* (Figure 5-11) or *Clostridium spp.* (Figure 5-12) isolated from caecal liquor following intravenous administration of amikacin. The number of streptococci isolated from ponies I1 and II2 (10^8-10^9/ml), prior to

Parameter	I1*	I2*	II1*	II1§	II2*
t1/2 B3 (h)	-	-	-	0.03	-
t1/2 B2 (h)	0.28	0.14	0.04	0.65	0.28
t1/2 B1 (h)	2.22	2.55	1.85	1.89	2.69
Cp0 (μg/ml)	56.13	60.90	75.85	78.42	61.04
Vc (ml/kg)	106.89	98.52	79.11	76.51	98.30
AUC_{obs} (μg.h/ml)	110.86	139.60	107.53	107.58	126.52
$AUMC_{obs}$ (μg.h^2/ml)	324.69	559.32	283.74	283.75	444.13
AUC (μg.h/ml)	106.72	137.47	102.99	102.79	123.83
AUMC (μg.h^2/ml)	312.87	460.63	269.63	268.14	434.79
MRT (h)	2.93	4.01	2.64	2.64	3.51
Vd_{area} (ml/kg)	180.42	169.15	155.34	158.73	188.13
Vd_{ss} (ml/kg)	164.84	162.37	152.52	152.26	170.12
CL_b (ml/h.kg)	56.23	45.99	58.26	58.67	48.45
k_{el} (/h)	0.53	0.47	0.74	0.76	0.49
k_{21} (/h)	1.47	2.83	10.14	12.16	1.29
k_{12} (/h)	0.80	1.84	9.41	10.87	0.94
k_{31} (/h)	-	-	-	0.99	-
k_{13} (/h)	-	-	-	0.10	-

Table 5-3. Disposition kinetics of amikacin in plasma following intravenous administration to ponies

Key: * calculated from a bi-exponential equation; § calculated from a tri-exponential equation

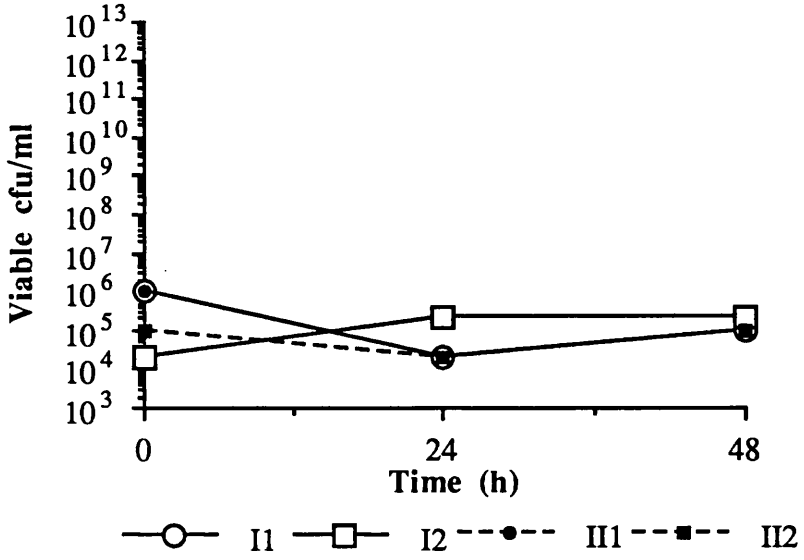

Figure 5-8. Counts of viable coliforms in caecal liquor following intravenous administration of amikacin to ponies

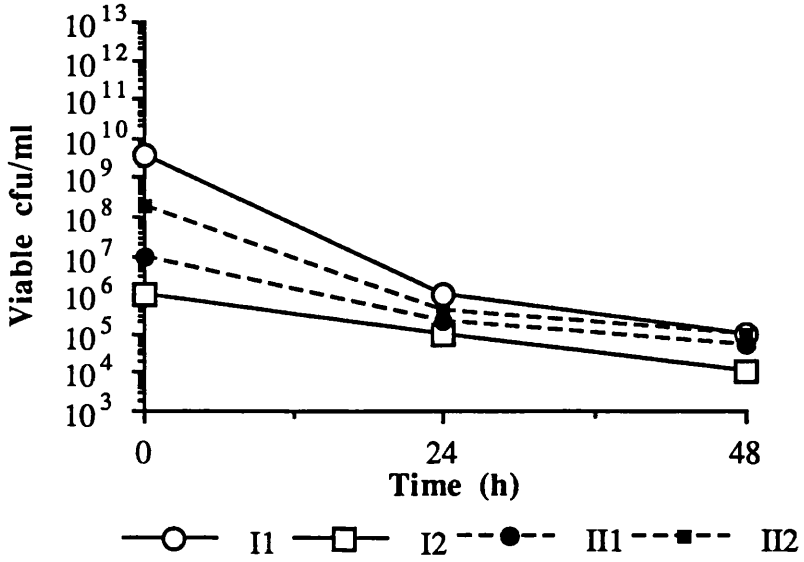

Figure 5-9. Counts of viable streptococci in caecal liquor following intravenous administration of amikacin to ponies

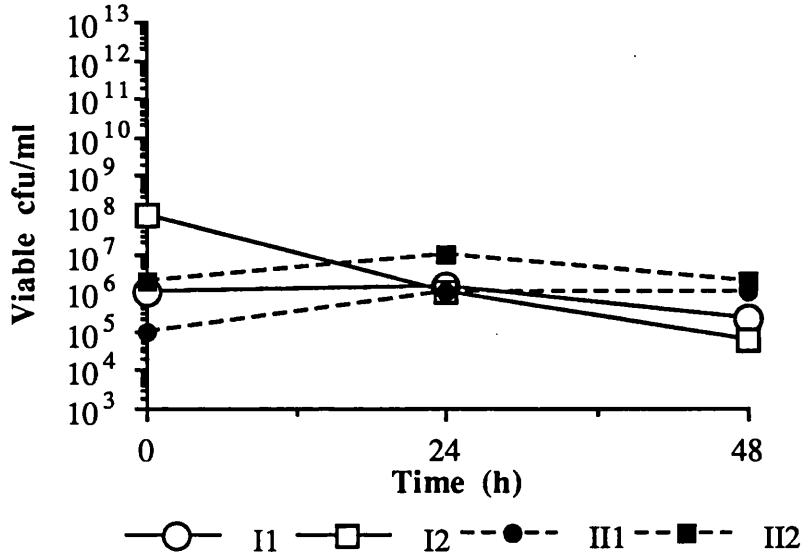

Figure 5-10 Counts of viable lactobacilli in caecal liquor following intravenous administration of amikacin to ponies

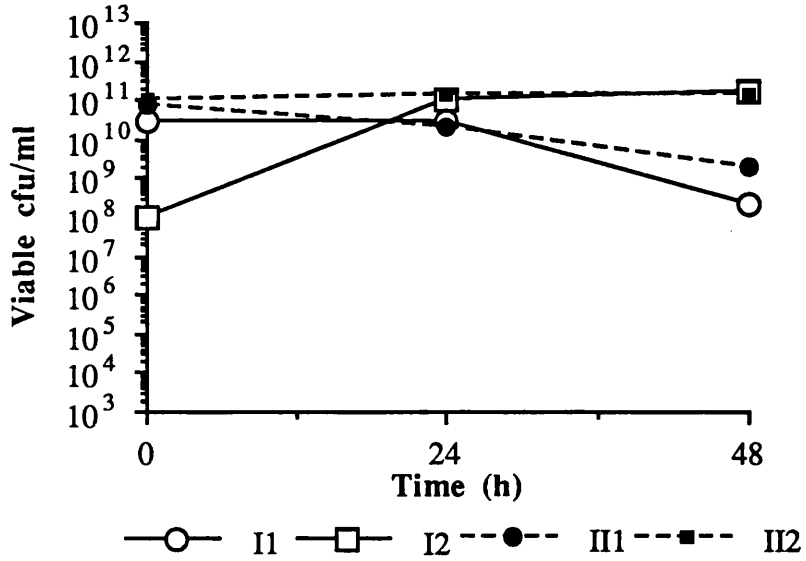

Figure 5-11. Counts of viable *Bacteroides spp.* in caecal liquor following intravenous administration of amikacin to ponies

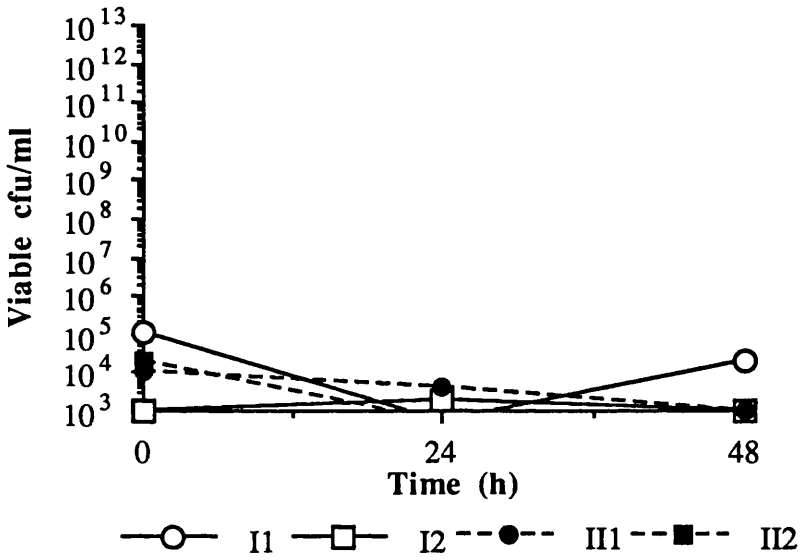

Figure 5-12. Counts of viable *Clostridium spp.* in caecal liquor following intravenous administration of amikacin to ponies

intravenous administration of amikacin, was higher than expected ($\leq 10^7$/ml). The number of *Bacteroides spp.* isolated from ponies I1 and I2 was low (10^8/ml) at 48 h after and prior to drug administration, respectively. Bacteria identified using the API system were *B. diastonis*, *Bacteroides spp. (B. ovatus/thetaiotaomicron)* and *C. butyricum.*

5.4.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

Caecal liquor pH values following intravenous administration of amikacin are shown in Figure 5-13, and the individual data is given in Appendix C (Table C26). Caecal liquor pH was slightly low (6.6) prior to drug administration in ponies I1 and I2 and remained between pH 6.7 and 7.2 for the remainder of both studies.

Caecal liquor lactic acid concentrations following intravenous administration of amikacin to ponies are shown in Figure 5-14, and the individual data is given in Appendix C (Tables C27a-C30a). There were no marked fluctuations in lactic acid concentrations following intravenous administration of amikacin. Lactic acid concentrations increased to 5.7-8.9 mmol/l (normal range 0.0-24.4 mmol/l) at 4 and 6 h after drug administration to pony I1.

Caecal liquor total VFA concentrations are shown in Figure 5-15, and the total VFA and individual acid concentrations are given in Appendix C (Tables C27a-C30a). There were alterations in VFA concentrations within the established normal ranges. However, there were very few VFA concentrations outside the normal ranges. In pony I2, the total VFA concentration of 131.3 mmol/l at 12 h after drug administration was higher than the normal range of 24.4-109.2 mmol/l, due to a butyric acid concentration of 68.7 mmol/l, compared with the normal range of 4.8-67.3 mmol/l.

The proportions of acetic, propionic and butyric acids, as a percentage of the total VFA concentrations, are given in Appendix C (Tables C27b, C28b, C29b and C30b). The ratios of acetic, propionic and butyric acids fluctuated around the normal ranges.

Faecal SCFA concentrations in ponies following intravenous administration of amikacin are shown in Figures 5-16 and 5-17, and the individual data is given in Appendix C (Tables C31-C34). Lactic acid concentrations were within the normal range of 0.0-24.4 mmol/l at all sample times in all ponies (Figure 5-16). The total VFA concentrations in faeces following intravenous administration of amikacin to ponies were very variable (Figure 5-17). There were variations in the total and individual VFA concentrations around the normal ranges. The total VFA concentration was lower than the normal range of 24.4 -109.2 mmol/l at 24 h in pony I1, at 0 and 48 h in pony I2, at 48 h in pony II1, and at 48 h in pony II2. Prior to drug administration, the total VFA concentration of 182.3 mmol/kg was higher than normal

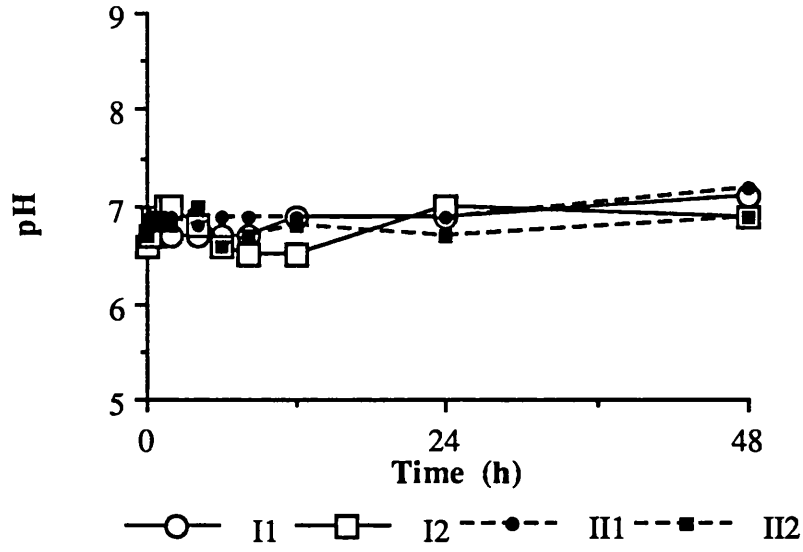

Figure 5-13. Caecal liquor pH following intravenous administration of amikacin to ponies

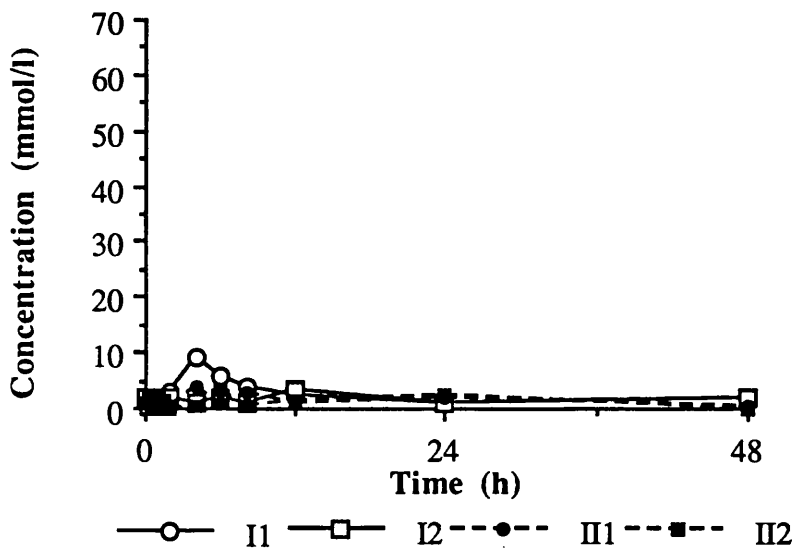

Figure 5-14. Lactic acid concentrations in caecal liquor following intravenous administration of amikacin to ponies

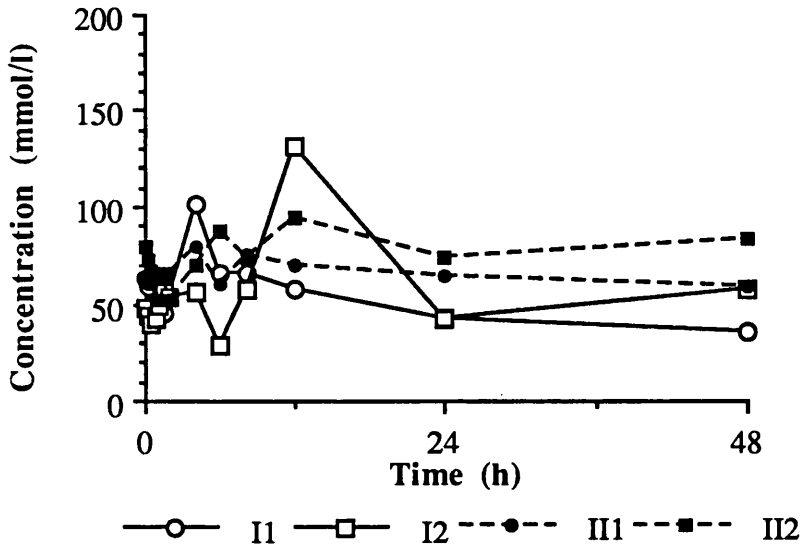

Figure 5-15. Total VFA concentrations in caecal liquor following intravenous administration of amikacin to ponies

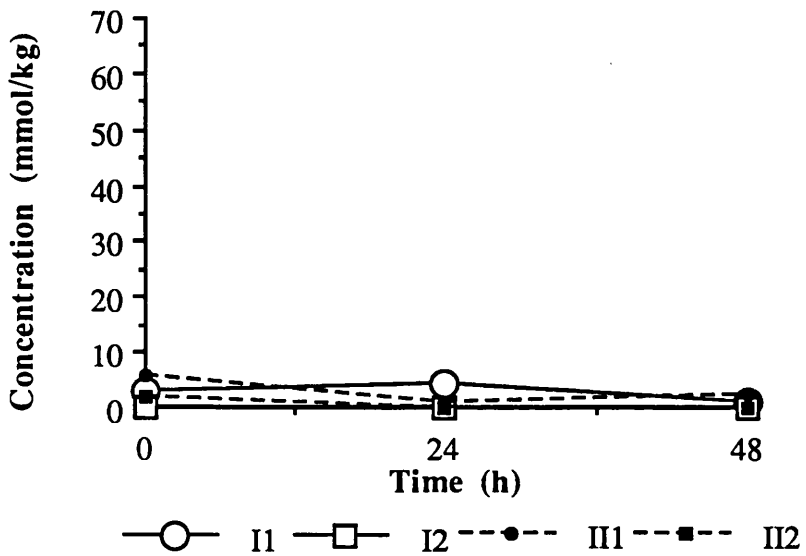

Figure 5-16. Lactic acid concentrations in faeces following intravenous administration of amikacin to ponies

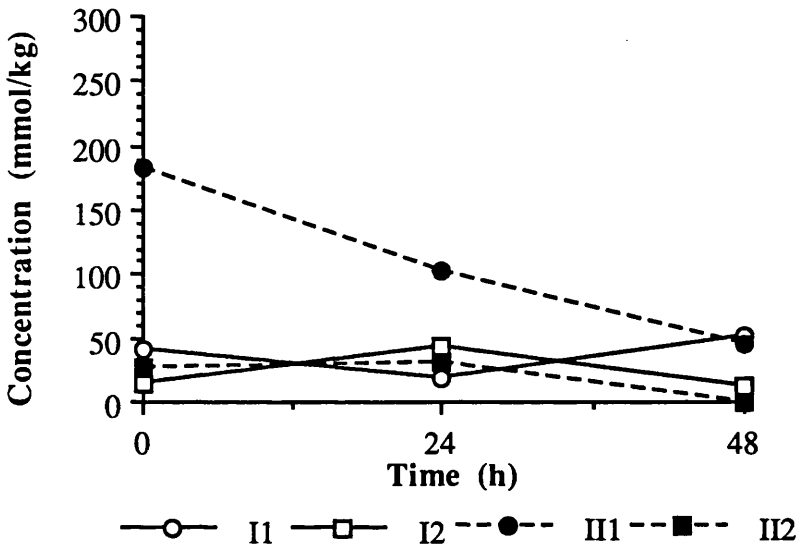

Figure 5-17. Total VFA concentrations in faeces following intravenous administration of amikacin to ponies

in pony II1 due to a very high butyric acid concentration of 179.3 mmol/kg, compared with the normal range of 4.8-67.3 mmol/l.

5.4.5 Faecal dry matter content and consistency

The faecal dry matter content following intravenous administration of amikacin to ponies is shown in Figure 5-18, and the individual data is given in Appendix C (Table C35). There were no marked alterations in faecal dry matter content and no changes in faecal consistency were observed.

5.4.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix C (Tables C36 and C37) and the results of the haematological examinations are given in Appendix C (Tables C38 and C39). There were no alterations in the plasma biochemistry or haematology values that were considered to be associated with the intravenous administration of amikacin to ponies.

5.5 Results of oral administration of amikacin to ponies with cannulated caecal fistulas

5.5.1 Plasma disposition and pharmacokinetics

The plasma concentrations of amikacin were below the limit of detection of the assay (<0.02 μg/ml) following oral administration to ponies.

5.5.2 Caecal liquor and faecal concentrations

The caecal liquor concentrations following oral administration of amikacin to ponies are shown in Figure 5-19, and the individual data is given in Appendix C (Table C40). Maximum caecal liquor concentrations of 99.37, 83.68, 21.65 and 16.19 μg/ml were measured at 2, 1.5, 8 and 12 h in ponies I1, I2, II1 and II2, respectively.

The disposition of amikacin in the caecal liquor was described using the observed AUC and AUMC to calculate the MRT (Table 5-4). There was a marked difference in the MRT between the 2 animals, a fact that was also reflected in the difference in the time to maximum caecal liquor concentrations.

Faecal concentrations following oral administration of amikacin to ponies are shown in Figure 5-20, and the individual data is given in Appendix C (Table C41).

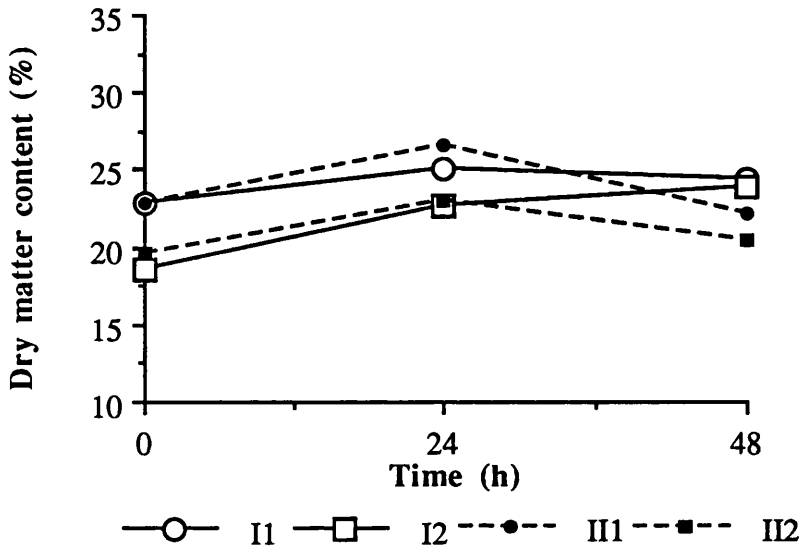

Figure 5-18. Faecal dry matter content following intravenous administration of amikacin to ponies

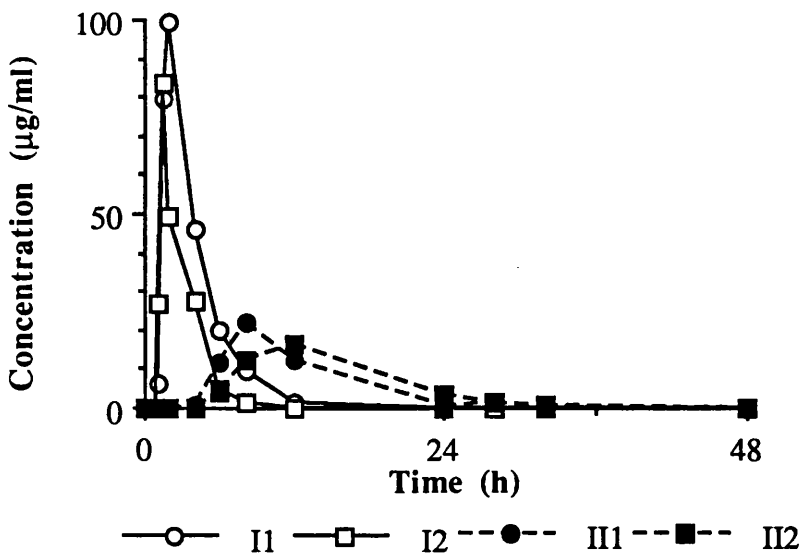

Figure 5-19. Caecal liquor concentrations of amikacin following oral administration to ponies

Parameter	I1	I2	II1	II2
AUC_{obs} (μg.h/ml)	335.94	182.41	204.40	215.07
$AUMC_{obs}$ (μg.h^2/ml)	1255.89	520.18	2268.44	2928.48
MRT (h)	3.74	2.85	11.10	13.62

Table 5-4. Disposition kinetics of amikacin in caecal liquor following oral administration to ponies

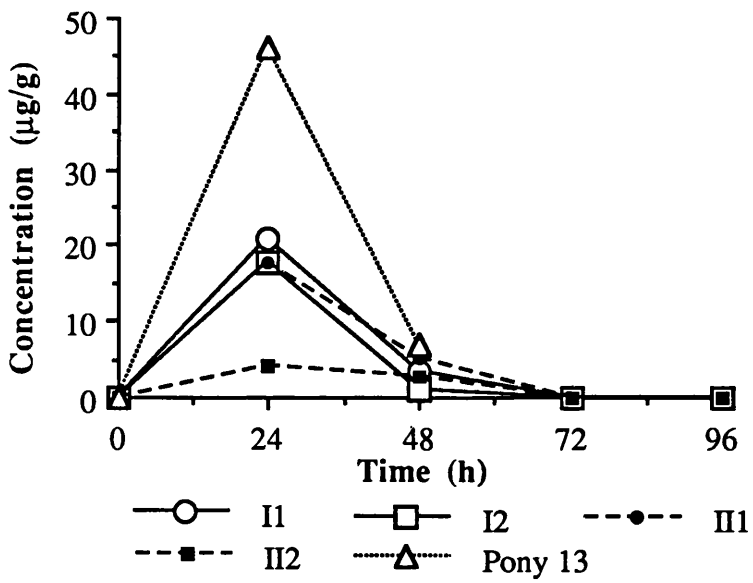

Figure 5-20. Faecal concentrations of amikacin following oral administration to ponies

5.5.3. Bacteriological examinations

Salmonella spp. and *C. difficile* were selected for but not isolated.

Counts of viable bacteria in caecal liquor following oral administration of amikacin are shown in Figures 5-21 to 5-26, and the individual data is given in Appendix C (Table C42).

There were no marked alterations in the number of coliforms isolated following oral administration of amikacin to ponies (Figure 5-21). There was an increase (10^9/ml) in the viable coliform counts at 96 h after drug administration to pony I1. There were slight increases (up to 10^7/ml) in the number of viable coliforms isolated from pony I2 at 72 and 96 h after drug administration and from pony II2 at 72 h after drug administration.

There were slight increases (10^7-10^8/ml) in the number of viable caecal streptococci isolated from pony I1 at 48, 72 and 96 h, from pony I2 at 24 h and from pony II1 at 24 and 72 h following oral administration of amikacin (Figure 5-22).

There was a moderate increase in the number of viable lactobacilli isolated following oral administration of amikacin (Figure 5-23). These were increased slightly to 10^7-10^8/ml at 24 and 168 h in pony I2 and at 96 h in ponies II1 and II2.

There were no marked alterations in the number of viable *Bacteroides spp.* isolated from ponies following oral administration of amikacin (Figure 5-24). There were low numbers of *Bacteroides spp.* (10^7-10^8/ml) isolated from pony I1 at 168 h, and from pony I2 prior to and 48 h after drug administration.

There were no marked alterations in the number of *Clostridium spp.* isolated following oral administration of amikacin (Figure 5-25). There were relatively high numbers (10^6-10^7/ml) of *Clostridium spp.* isolated from ponies I1 and II1 prior to and from pony II1 at 48 h after drug administration. *Clostridium butyricum* was isolated and identified using the API system.

There was a marked increase in the number of viable faecal streptococci (up to 10^{10}/g) isolated from pony 13 at 24 and 48 h after drug administration (Figure 5-26; Appendix C, Table C42). The number of viable lactobacilli isolated from pony 13, at 24 and 48 h after drug administration, was high (10^8-10^9/g). There were no marked alterations in the number of *Bacteroides spp.* isolated from pony 13 following oral administration of amikacin, however low numbers (10^8/g) were isolated prior to drug administration. There were high numbers of *Clostridium spp.* (10^7/g) isolated from pony 13 at 48 h after drug

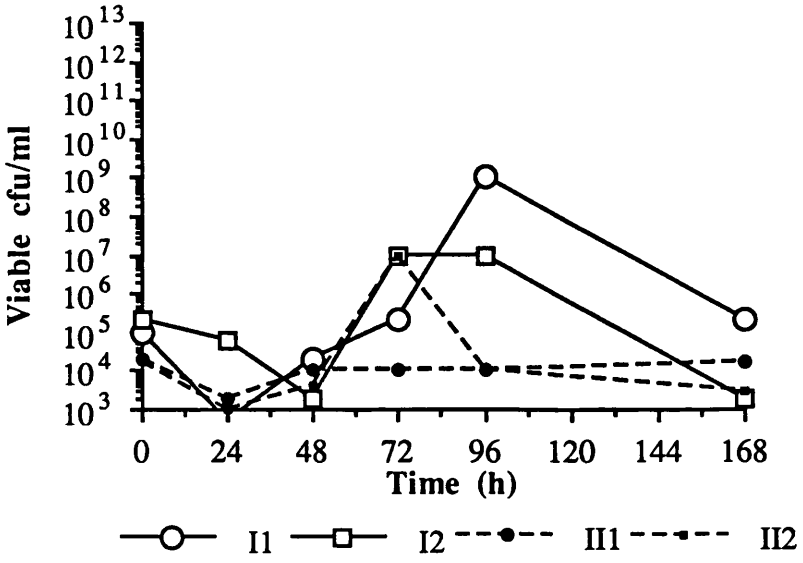

Figure 5-21. Counts of viable coliforms in caecal liquor following oral administration of amikacin to ponies

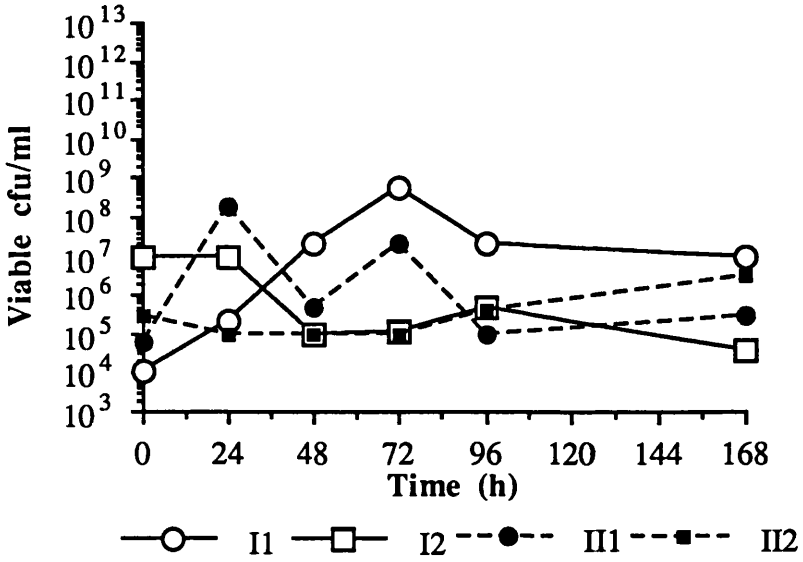

Figure 5-22. Counts of viable streptococci in caecal liquor following oral administration of amikacin to ponies

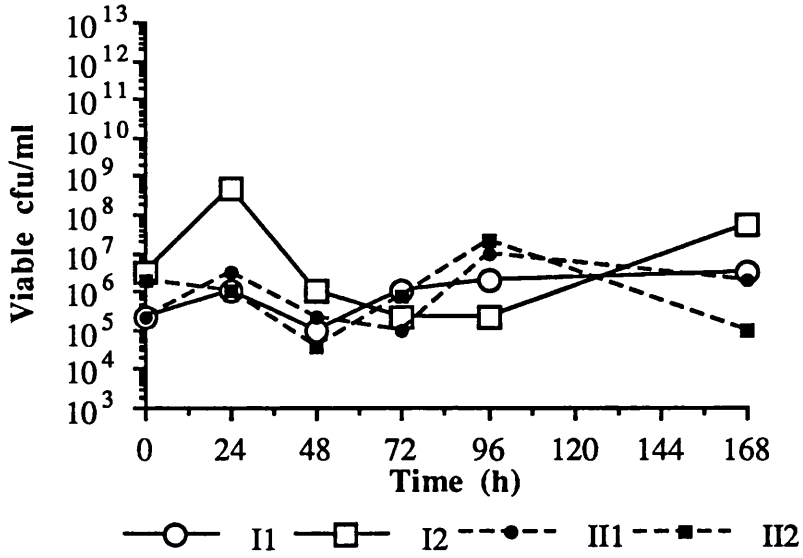

Figure 5-23. Counts of viable lactobacilli in caecal liquor following oral administration of amikacin to ponies

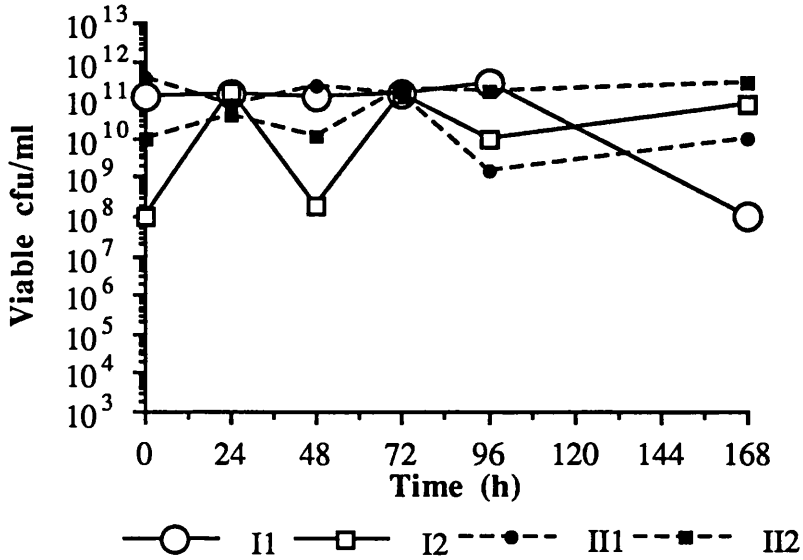

Figure 5-24. Counts of viable *Bacteroides spp.* in caecal liquor following oral administration of amikacin to ponies

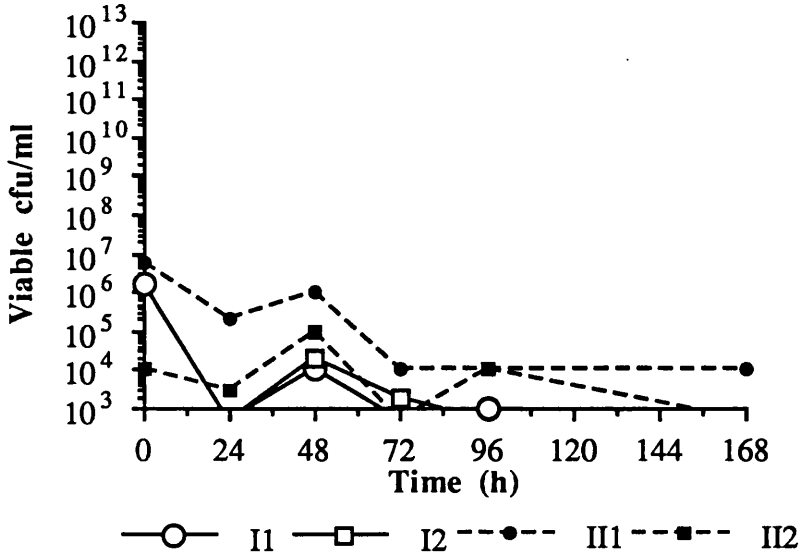

Figure 5-25. Counts of viable *Clostridium spp.* in caecal liquor following oral administration of amikacin to ponies

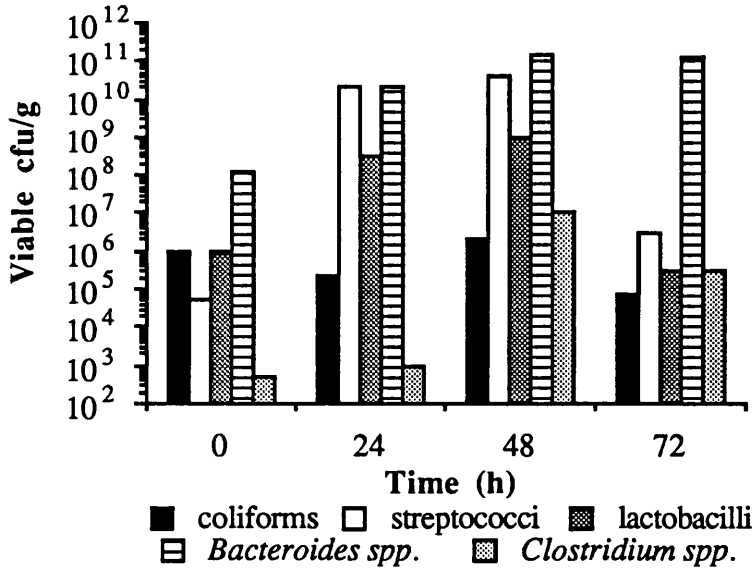

Figure 5-26. Counts of viable bacteria in faeces following oral administration of amikacin to pony 13

administration. *Clostridium perfringens* was isolated from pony 13 at 72 h after drug administration (10^4/g), and identified using the API system .

5.5.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

The caecal liquor pH measurements are shown in Figure 5-27, and the individual data is given in Appendix C (Table C43). There appeared to be a reduction in the caecal liquor pH following oral administration of amikacin. Caecal liquor pH fell to lower than pH 6.6 at 6, 8, 12, 28 and 32 h in pony I1, at 2, 4, 6, 8, 12, 28, 32, 48, 52 and 56 h in pony I2, at 12, 28 and 56 h in pony II1, and at 2, 4 and 6 h in pony II2.

Caecal liquor SCFA concentrations are shown in Figures 5-28 and 5-29, and the individual data is given in Appendix C (Tables C44a-C47a).

Caecal liquor lactic acid concentrations increased following the oral administration of amikacin although the majority of concentrations lay within the normal range of 0.0-24.4 mmol/l (Figure 5-28). In pony I1, lactic acid concentrations were elevated to 6.5-24.4 mmol/l at 2, 4, 6, 8, 12, 28, 52 and 56 h, and at 4 h lactic acid concentrations were outside the normal range. In pony I2, lactic acid concentrations were elevated to 13.0-28.8 mmol/l at 4, 6, 8, 12, 24, 28 and 32 h, and were outside the normal range at 6, 8 and 12 h after drug administration. Lactic acid concentrations were increased to 14.4-20.3 mmol/l at 12, 24 and 28 h in pony II1, and to 5.2-7.8 mmol/l at 24, 28 and 56 h in pony II2.

Total VFA concentrations did not alter markedly following oral administration of amikacin (Figure 5-29). There were very few VFA concentrations outside the normal ranges. The butyric acid concentration fell to 3.7 mmol/l, compared with the normal range of 4.8-67.3 mmol/l, at 6 and 12 h after drug administration to pony I2, and to 3.9 mmol/l at 0.75 h after drug administration to pony II2.

The proportions of acetic, propionic and butyric acids, as a percentage of the total VFA concentrations are given in Appendix C (Table C44b, C45b, C46b, C47b). The ratios of acetic, propionic and butyric acids were all within or around the normal ranges.

SCFA concentrations in faeces are shown in Figures 5-30 and 5-31, and the individual data is given in Appendix C (Tables C48-C52). There were no marked alterations in the lactic acid concentrations in faeces following oral administration of amikacin (Figure 5-30). Although, in pony II1 the lactic acid concentration was increased to 15.4 mmol/kg at 24 h, compared with normal range of 0.0-24.4 mmol/l. There were no marked alterations in the total VFA concentrations in faeces (Figure 5-31). Total VFA concentrations in faeces were

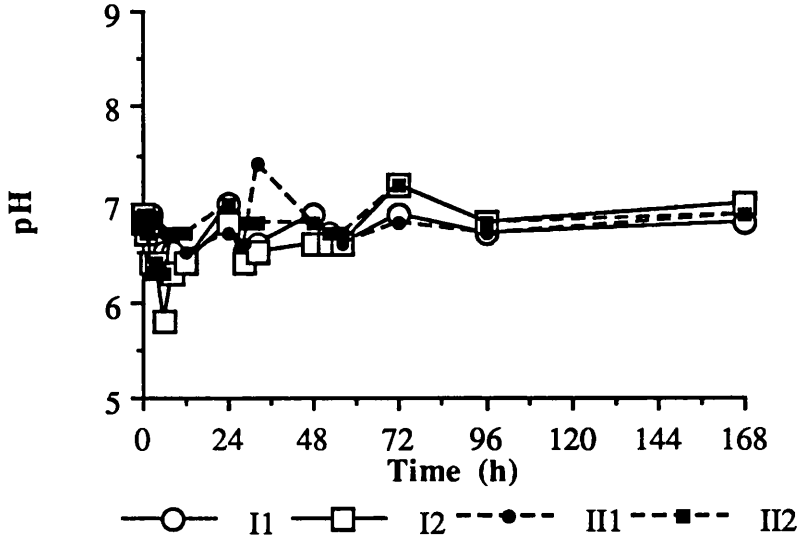

Figure 5-27. Caecal liquor pH following oral administration of amikacin to ponies

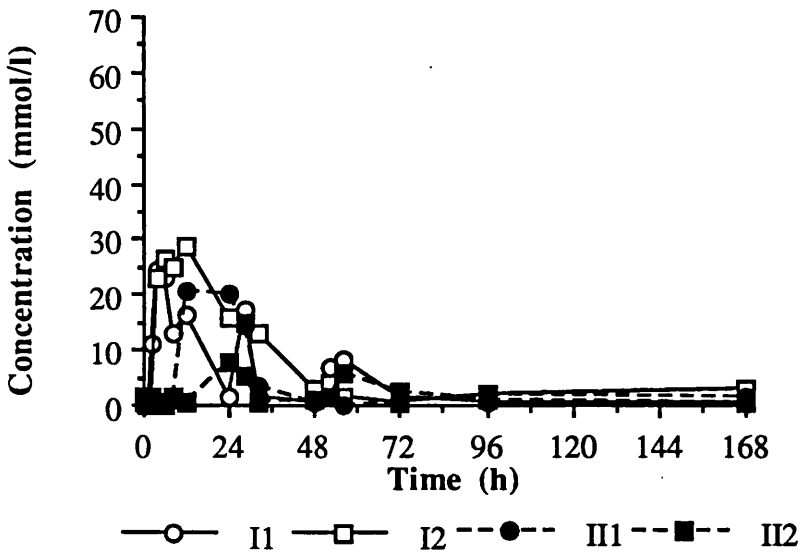

Figure 5-28. Lactic acid concentrations in caecal liquor following oral administration of amikacin to ponies

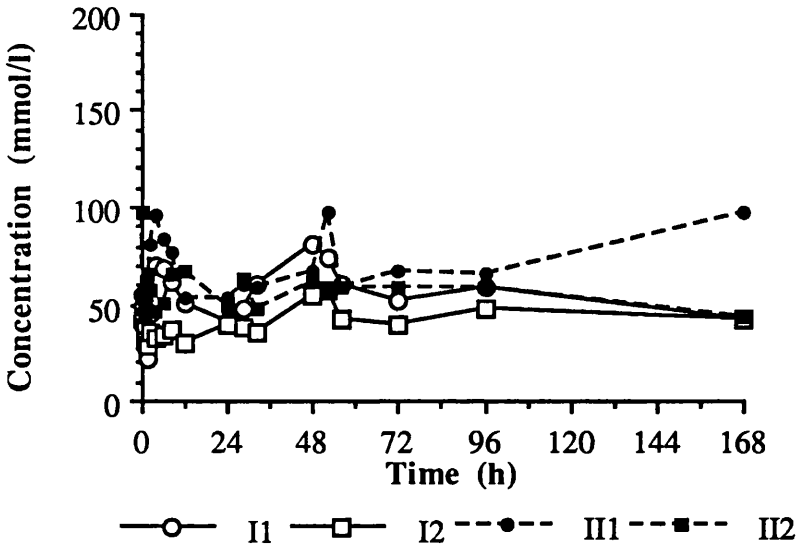

Figure 5-29. Total VFA concentrations in caecal liquor following oral administration of amikacin to ponies

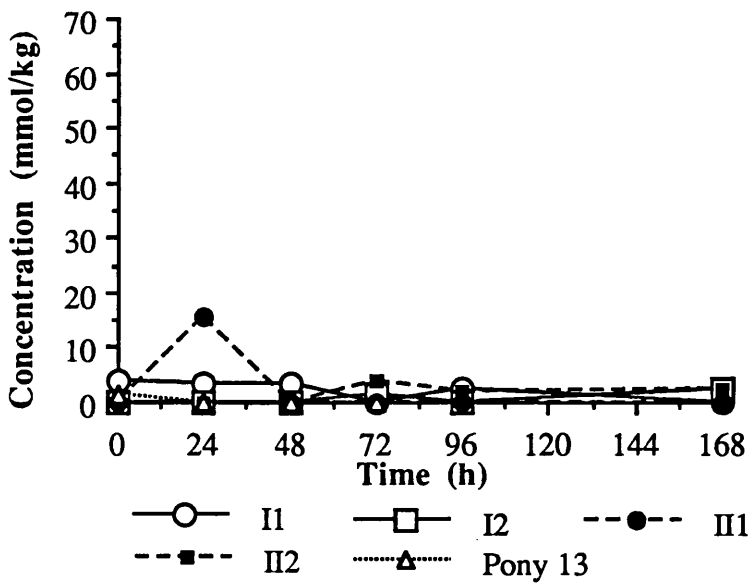

Figure 5-30. Lactic acid concentrations in faeces following oral administration of amikacin to ponies

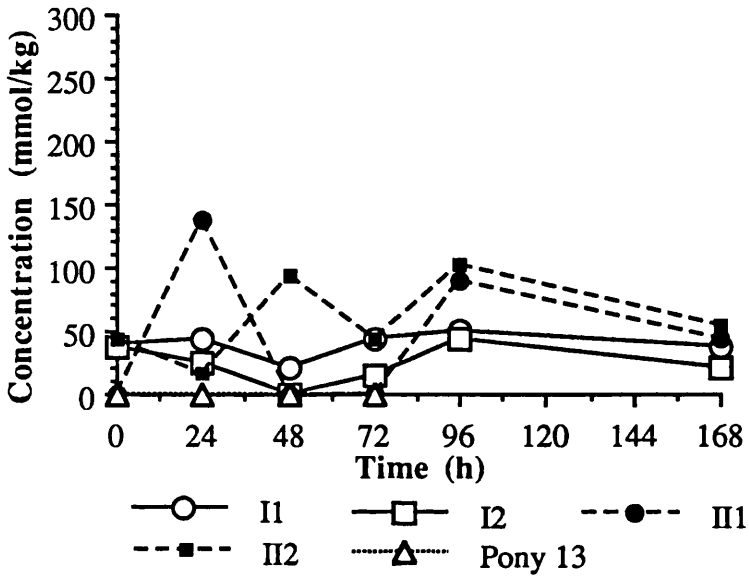

Figure 5-31. Total VFA concentrations in faeces following oral administration of amikacin to ponies

lower than the normal range of 24.4-109.2 mmol/l at 48 h in pony I1, at 48, 72 and 168 h in pony I2, at 0, 48 and 72 h in pony II1, at 24 h in pony II2 and throughout the study in pony 13. In addition, the total VFA concentration was higher than normal at 24 h after drug administration to pony II1. There were alterations in individual VFA concentrations, but there were no apparent trends in VFA concentrations that were associated with the oral administration of amikacin.

5.5.5 Faecal dry matter content and consistency

The faecal dry matter content following oral administration of amikacin is shown in Figure 5-32, and the individual data is given in Appendix C (Table C53). There were no marked changes observed in either the faecal dry matter content or the faecal consistency following the oral administration of amikacin to ponies.

5.5.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix C (Tables C54-C57) and the results of the haematological examinations are given in Appendix C (Tables C58-C61). There were no alterations in plasma biochemistry or haematology that were associated with the oral administration of amikacin.

5.6 Results of *in vitro* studies with amikacin

5.6.1 Caecal liquor concentrations

Concentrations (mean±SEM) of amikacin in caecal liquor following incubation *in vitro* for 3 and 24 h are shown in Figure 5-33, and the individual data is given in Appendix C (Tables C62a and b). After 3 h incubation, there was an average of 32% of amikacin remaining at the initial concentration of 0.25 μg/ml, whilst the majority of the drug remained at 1, 5, 10 and 20 μg/ml, and 55-75% of the drug remained at 40 and 80 μg/ml. The largest reductions in concentration (zone diameter) were seen at either end of the concentration range due to limitations of the assay technique (zone diameter, limit of detection). There was an average of 90.8% of the drug activity remaining after 3 h incubation. Following 24 h incubation, the percentage reduction in concentration in all the samples was similar to that seen at 3 h, except there were no inhibition zones for the 0.25 μg/ml sample, and there was only 63% of the 1 μg/ml sample remaining. There was an average of 85.0% (excluding the 0.25 μg/ml sample) of the drug activity remaining following 24 h incubation.

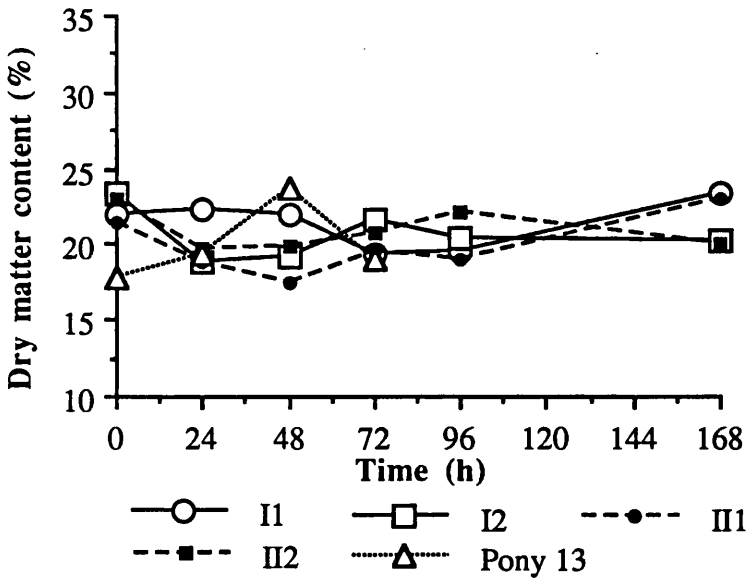

Figure 5-32. Faecal dry matter content following oral administration of amikacin to ponies

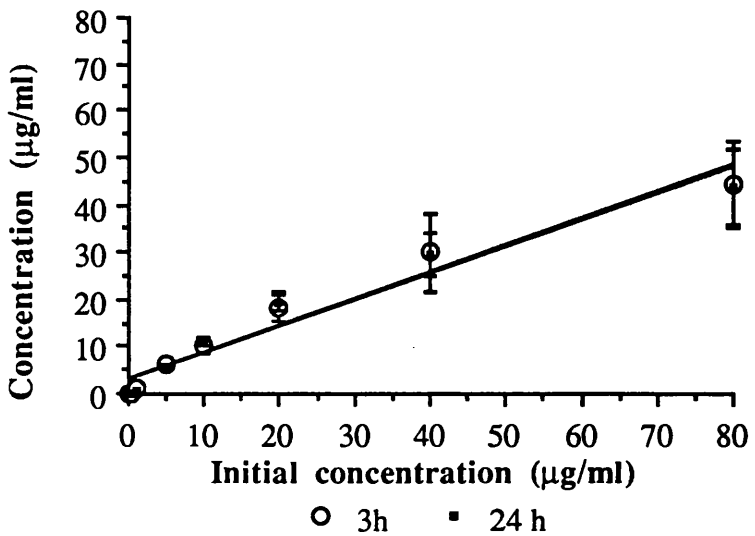

Figure 5-33. Caecal liquor concentrations (mean±SEM) of amikacin following incubation *in vitro* for 3 and 24 h

5.6.2 SCFA concentrations

Caecal lactic acid and total VFA concentrations (mean±SEM) following *in vitro* incubation are shown in Figure 5-34, and the individual data is given in Appendix C (Tables C63-C67). There were no alterations in mean lactic acid or mean total VFA concentrations with time or drug concentration.

5.6.3 Acid pH

Amikacin concentrations (mean±SEM) remaining following *in vitro* incubation at pH 1.9 are shown in Figure 5-35, and the individual data is given in Appendix C (Table C68). There was very little amikacin destroyed by incubation at pH 1.9; on average 85.6% of the drug remained after 1 h incubation. There may be some variation in percentage of drug destroyed with concentration; 87.0, 100.0, 84.6 and 70.9% of drug remained (mean) at 1, 2, 5 and 10 µg/ml respectively.

5.6.4 Binding to hay

Amikacin concentrations remaining following *in vitro* incubation with chopped hay at pH 1.9 and pH 7.0 for 3 h are shown in Figure 5-36. There was only 1 replicate of this study performed. There was a range of the initial 10, 25, 50 and 100 µg/ml amikacin remaining following incubation, namely 0.33, 1.35, 2.40, and 8.81 µg/ml at pH 1.9, and 0.23, 0.65, 2.47 and 6.76 µg/ml at pH 7.0. However, it is interesting that only 3.3, 5.4, 4.8 and 8.8% remained at pH 1.9, and 2.3, 2.6, 5.0 and 6.8% at pH 7.0, or an average of 5.6% and 4.2%, respectively.

5.7. Discussion

In the present study, following intravenous administration of amikacin at a dose rate of 6 mg/kg bwt to horses, ponies and donkeys, the elimination half-lives were 2.84, 1.60 and 1.93 h (harmonic mean), respectively. In the present study, the elimination half-life of amikacin in horses was longer than in ponies and donkeys. Brown *et al.* (1984c) reported that the disposition of amikacin in plasma following intramuscular administration at a dose rate of 7 mg/kg bwt was best described by a bi-exponential equation with a mean elimination half-life of 2.3 h. Similarly, the mean elimination half-life of amikacin following intramuscular administration to foals was 3 h (Brown *et al.*, 1986). Orsini *et al.* (1985) calculated elimination half-lives (harmonic mean) of 1.44, 1.57 and 1.14 h following intravenous administration of amikacin at dose rates of 4.4, 6.6 and 11 mg/kg bwt, respectively. In the present study the CL_b (mean±SEM) following intravenous administration to horses, ponies and donkeys was 45.19±3.76, 82.37±11.27 and

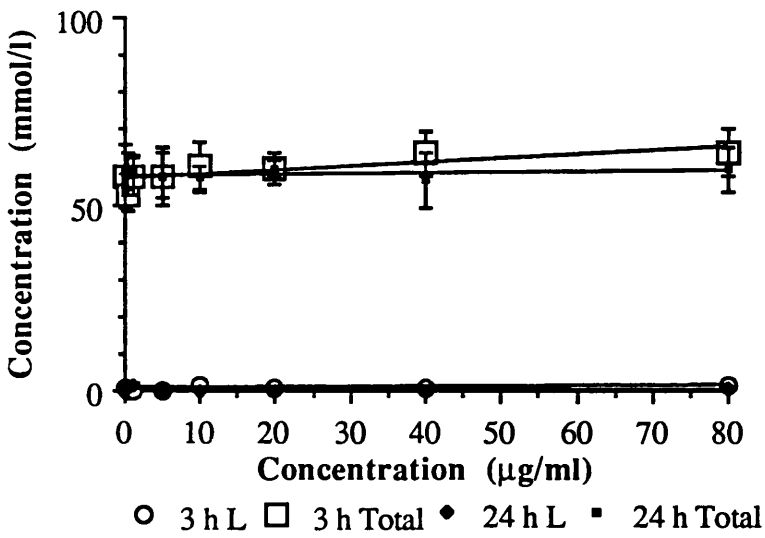

Figure 5-34. SCFA concentrations (mean±SEM) in caecal liquor following incubation *in vitro* with amikacin for 3 and 24 h

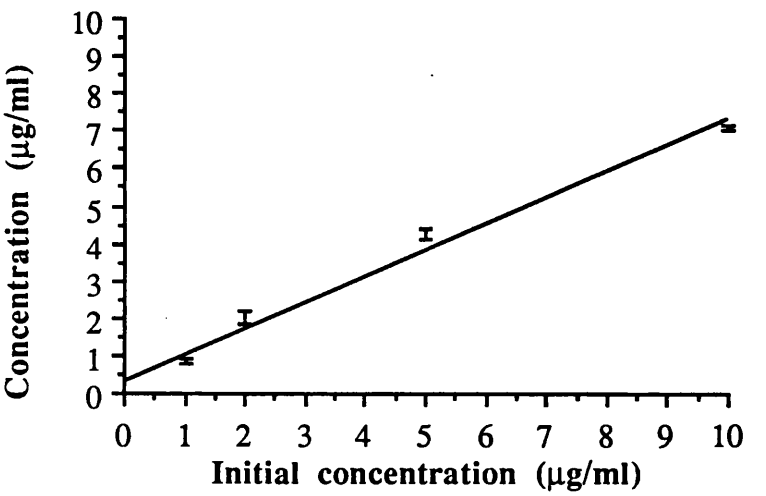

Figure 5-35. Concentrations (mean±SEM) of amikacin following incubation *in vitro* at pH 1.9 for 1 h

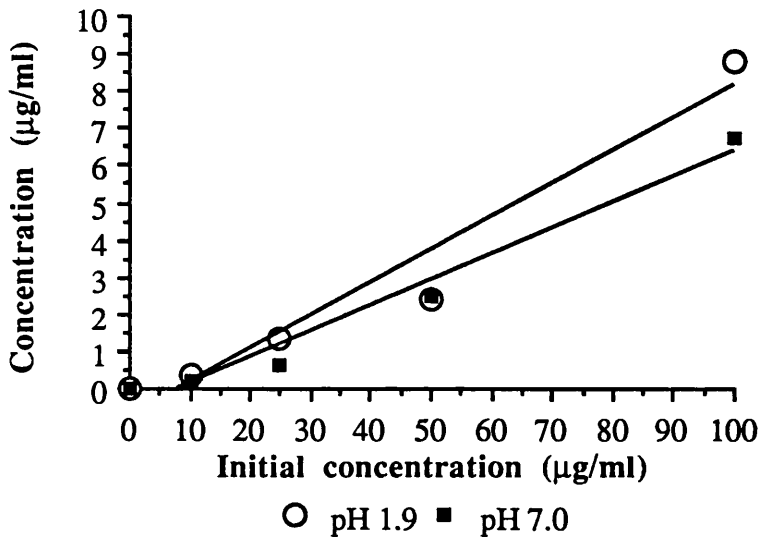

Figure 5-36. Concentrations of amikacin following *in vitro* incubation with hay at pH 1.9 and pH 7.0 for 3 h

57.98±15.05 ml/h.kg, respectively, whereas in a previous study it was 76.6±11.3 ml/h.kg (mean±SD) following intravenous administration of amikacin to horses at a dose rate of 6.6 mg/kg bwt (Orsini *et al.*, 1985). Other pharmacokinetic parameters calculated in the present study were similar to those calculated in previous studies.

An optimum drug dose requires that the tissue concentrations of the drug equal or exceed the MIC of susceptible pathogens (Prescott and Baggot, 1985). Bacterial killing is directly proportional to the drug concentrations for the aminoglycosides (Prescott and Baggot, 1985). Generally, the MIC of a susceptible Gram positive or Gram negative bacterium, including *Pseudomonas aeruginosa*, is ≤4 µg/ml amikacin (Baggot and Prescott, 1987, Orsini *et al.*, 1985). Hirsch and Jang (1987) reported that 94% of coagulase-positive staphylococci isolated from the skeletal system of horses were susceptible *in vitro* to 4 µg/ml amikacin and this increased to 100% at 8 µg/ml. However, isolates of *Streptococcus zooepidemicus* from the lower respiratory tract of horses were more resistant (23% at 4 µg/ml, 100% at 64 µg/ml). In addition, 87% of isolates of *Corynebacterium* (*Rhodococcus*) *equi* were susceptible to 2 µg/ml amikacin, 98% at 4 µg/ml, and 100% were susceptible to amikacin at a concentration of 8 µg/ml, *in vitro*. Although, clinical isolates of *Bacteroides spp.* and *Klebsiella pneumoniae* and *Salmonella typhimurium* were susceptible *in vitro* to ≤4 µg/ml amikacin, isolates of *E. coli* appeared to be more resistant (93% at 4 µg/ml, 100% at >64 µg/ml) *in vitro* (Hirsch and Jang, 1987). In the present study, the mean plasma concentrations of amikacin were greater than or equal to 4 µg/ml for 8 h in horses, and 6 h in ponies and donkeys following a single intravenous administration at a dose rate of 6 mg/kg bwt. Similarly, Orsini *et al.* (1985) reported that mean serum concentrations were maintained above 4 µg/ml for 8 h, following intravenous administration of amikacin at dose rates of 6.6 and 11.0 mg/kg bwt, and for around 5 h, following intravenous administration at a dose rate of 4.4 mg/kg bwt. This would suggest that intravenous administration of amikacin at a dose rate of 6 mg/kg bwt should be repeated every 8 h in horses and every 6 h in ponies and donkeys. This is similar to the recommendation of Van Dyk *et al.* (1982) who suggested that the intravenous administration of amikacin should be repeated every 6-8 h to maintain therapeutic plasma concentrations. However, Orsini *et al.* (1985) concluded that amikacin attained therapeutic concentrations in serum, peritoneal and synovial fluid and that intravenous administration at a dose rate of 4.4-6.6 mg/kg bwt every 12 h would provide suitable therapeutic plasma concentrations for treating susceptible infections, but for more serious, life-threatening infections, dosing three times daily was recommended.

It has been shown that at concentrations below the MIC, or the minimum bactericidal concentration, antimicrobial agents still show some inhibitory effects on the growth of bacteria, *in vitro*. The minimum bactericidal concentrations, for 10 clinical specimens of *E.*

coli, were 4.0-40.0 μg/ml amikacin and the minimum antibiotic concentration, based on two-fold broth dilutions and post-incubation counts of viable cfu, was $^1/_4$-$^1/_{32}$ of the minimum bactericidal concentrations for gentamicin and amikacin (Zanon, 1977). If the minimum antibiotic concentration has relevance *in vivo* then amikacin would need to be repeated less frequently, *e. g.* twice daily at a dose rate of 6 mg/kg bwt. This is of particular importance when an antimicrobial agent with a low therapeutic index is used.

There was no amikacin detected in caecal liquor or faeces following intravenous or oral administration. Snyder *et al.* (1986) measured peak concentrations of gentamicin in the tissue of the jejunum and large colon with peak concentrations of 2.26±1.35 μg/ml at 0.33 h and 4.13±1.80 μg/ml at 0.5 h, respectively, following intravenous administration of gentamicin. This is not surprising since the blood flow to the intestinal wall is quite high. However, the aminoglycosides are polar and have an apparent volume of distribution similar to the extracellular fluid volume of the animal. In the present study, the mean rate of distribution of amikacin to the hypothetical second compartment (k_{12}), representing well perfused tissues, was similar to the mean rate of elimination from this compartment (k_{21}). Hence the predicted penetration of well-perfused tissues appeared to be poor. However, therapeutic concentrations of amikacin and gentamicin have been measured in peritoneal and synovial fluid (Bowman *et al.*, 1986, Orsini *et al.*, 1985), and amikacin has been used successfully to treat respiratory infections (Cudd, 1985). This suggests that the distribution of these polar antimicrobial agents is wider *in vivo* than it appears in pharmacokinetic models.

In the present study, it seems unlikely that amikacin was eliminated in bile to any great extent following intravenous administration, since the caecal liquor concentrations remained below the limit of detection of the assay (0.03 μg/ml). Aminoglycosides are excreted unchanged by the kidney but Ziv *et al.* (1982) reported mean concentrations of 22.0, 12.5 and 8.2 μg/ml in bile at 4, 8 and 12 h after intravenous administration of gentamicin to calves. However, the concentration of free gentamicin in bile was <7% of the simultaneous urine concentrations and was <0.2% of the administered dose. The presence of biliary concentrations of an aminoglycoside may reflect the high concentrations present in the well-perfused hepatic tissue following intravenous administration. However, Ziv *et al.* (1982) reported that free concentrations of gentamicin in the liver were ≤1.2 μg/ml at 4, 8 and 12 h after intravenous administration and that this represented ≤0.86% of the administered dose. The primary event in bile formation is the active secretion of bile salts, and this is accompanied by a passive flow of electrolytes and water. It seems more likely that gentamicin was present in bile through movement along with the aqueous phase of bile, since the concentrations discussed in this study were measured by bioassay, and represented free drug. The situation that

occurs in the horse may be totally different since the horse has no gall bladder and the bile flow into the duodenum tends to be more or less continuous.

There were no marked changes in the number of viable bacteria isolated from caecal liquor or faeces following intravenous or oral administration of amikacin. There was a modest increase in the number of coliforms, streptococci and lactobacilli, and possibly *Clostridium spp.*, isolated following oral administration of amikacin. The absence of marked alterations in the numbers of viable caecal luminal bacteria following oral administration should not have been particularly surprising since the aminoglycosides are of no value in an anaerobic environment (Brumbaugh, 1987). However, the aminoglycosides are used to sterilize the large intestine prior to surgery. In man, a one day dosage regime has been used to produce intra-operative suppression of the gastrointestinal flora and a lengthy 3 or 5 day pre-operative regime is considered to be unnecessary (Gorbach, 1983). However, in man the administration of antimicrobial agents prior to intestinal surgery is combined with administration of a low residue diet, magnesium sulphate and saline enemas. In addition, the estimated luminal concentrations of neomycin following oral administration were 1-3 mg/ml, whereas in the present study the peak caecal liquor concentrations of amikacin were 0.1, 0.08, 0.02 and 0.02 mg/ml in ponies I1, I2, II1 and II2 following oral administration. Also, the neomycin oral bowel preparation regime utilizes concomitant administration of erythromycin to achieve the desired effects (Gorbach, 1983).

There was a reduction in caecal liquor pH following the oral administration of amikacin which may be explained by the increases in lactic acid concentrations. The number of viable lactic acid producing bacteria, such as streptococci, lactobacilli and *Clostridium spp.*, increased slightly following oral administration. The peak lactic acid concentrations in the present study occurred at variable times following the oral administration of amikacin, although they always occurred following the peak drug concentrations. Similarly, the reduction in caecal liquor pH followed peak drug concentrations. However, the trough pH measurements did not occur at the same time as the peak lactic acid concentrations. It is interesting that there were practically no VFA concentrations outside the established normal ranges despite the large number of potentially susceptible Gram negative bacteria in the gastrointestinal lumen.

There were no marked alterations in plasma urea or creatinine concentrations in the present study. There have been reports of nephrotoxicity following the administration of gentamicin in the equine. Riviere *et al.* (1983b) reported clinical signs of nephrotoxicity (increased blood urea nitrogen and plasma creatinine concentrations) following the administration of gentamicin to foals and suggested that young horses may be more susceptible to gentamicin toxicity. Similarly, Riviere *et al.* (1982) recorded gentamicin nephrotoxicity at recommended

dose rates in three horses, although in one case it was reversed by drug withdrawal and volume diuresis. Sweeney *et al.* (1988) reported gentamicin induced acute renal failure following administration at a dose rate of 2.2 mg/kg every 6 h, and Hinchcliff *et al.* (1989) induced nephrotoxicosis in ponies by the administration of gentamicin at a dose rate of 20 mg/kg bwt for up to 14 days. Following intravenous administration of amikacin to horses at a dose rate of 10 mg/lb (6.8 mg/kg) bwt daily for 15 days there was no clinical, laboratory or histopathological evidence of ototoxicity, nephrotoxicity or other adverse effects (Gingerich *et al.*, 1983). Similarly, Adland-Davenport *et al.* (1990) noted no recognizable signs of amikacin-induced nephrotoxicosis following administration of amikacin to critically ill neonatal foals, and Cudd (1985) reported that intramuscular administration of amikacin at ≤11 mg/kg bwt every 8 h for up to 4 weeks did not result in the impairment of renal function in horses. The aminoglycosides bind to the brush border of the tubular epithelium in the kidney and translocate into the cytoplasm and lysosomes of cells resulting in lysosomal disruption and tubular necrosis (Davis, 1987). The nephrotoxic potential of the aminoglycosides appears to be dose-related (Brumbaugh, 1987, Davis, 1987) and in man, trough plasma concentrations of gentamicin of >2 μg/ml have been more closely associated with the development of nephrotoxicity than high peak concentrations (Brumbaugh, 1987). In view of this, it would be advisable to allow plasma concentrations to fall below 2 μg/ml in the inter-dosing interval. In addition, monitoring of plasma concentrations and indicators of renal function during therapy is useful when agents with a low therapeutic index, such as the aminoglycosides, are used for therapy (Brumbaugh, 1987).

The present study suggests that a suitable dosage regimen for the intravenous administration of amikacin sulphate to horses, ponies and donkeys, at a dose rate of 6 mg/kg bwt, would be every 12 h in horses, and every 8 h in ponies and donkeys. There appears to be no particular risk of development of enterocolitis following a single oral administration of amikacin sulphate, at a dose rate of 6 mg/kg bwt, to ponies. However, the luminal concentrations achieved in the present study may well be insufficient for suppression of the gastrointestinal flora. In addition, at a higher dose rate more extreme alterations in the number of viable bacteria, caecal liquor pH and lactic acid concentrations may result in the development of clinical changes associated with gastrointestinal acidosis.

6 Studies with oxytetracycline

6.1 Introduction

In the horse, plasma and serum concentrations of oxytetracycline have been determined following intravenous administration at dose rates of 2.5-5.0 mg/kg bwt (Baggot, 1977b, Brown *et al.*, 1981, Pilloud, 1973 and Teske *et al.*, 1973). There is little information on the plasma concentrations of oxytetracycline following intravenous administration at 10 mg/kg bwt, the dose rate recommended frequently in other species (Bywater, 1982b, Prescott and Baggot, 1988b and Van Miert, 1988).

Prescott and Baggot (1988b) noted that many veterinarians have used the tetracyclines in horses without observing adverse effects. However, the tetracyclines have been reported to cause diarrhoea in the horse, both in clinical cases (Manahan, 1970, Baker and Leyland, 1973, Mackellar *et al.*, 1973) and in experimental animals (Andersson *et al.*, 1971). It has been postulated that the intestinal flora of the horse is disturbed easily by the excretion of high concentrations of oxytetracycline in bile (Cook, 1973). Similarly, Swerczek (1979) reported that the use of antimicrobial agents, such as oxytetracycline, in healthy horses may alter the commensal gastrointestinal bacteria, and allow the overgrowth of bacterial pathogens such as *C. perfringens*. Therefore, it has been recommended that tetracycline therapy should be avoided in the equine due to the potential of inducing severe diarrhoea (Prescott and Baggot, 1988b).

The purpose of the present study was to determine the plasma concentrations of oxytetracycline hydrochloride in horses, ponies and donkeys after intravenous administration at the dose rate recommended for other species. Alterations in the intestinal flora were studied by bacteriological examination of serial faecal samples and large intestinal fermentation was studied by determining faecal SCFA concentrations. Faecal dry matter content and the gross appearance of faeces were used as indicators of the presence or absence of diarrhoea. Similar studies were carried out in ponies with cannulated caecal fistulas following a single intravenous or oral administration of oxytetracycline hydrochloride at a dose rate of 10 mg/kg bwt. In addition, drug, bacteriological and SCFA analyses were carried out on caecal liquor, and plasma biochemistry and haematology were monitored.

6.2 Materials and Methods

6.2.1 Intravenous administration of oxytetracycline to horses, ponies and donkeys

Three Thoroughbred geldings (No. 1-3), four ponies (No. 7-10) and three donkeys (No. 15-17) were used, as described in the general Materials and Methods. Oxytetracycline hydrochloride was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples for drug analysis were taken at 0, 0.083, 0.25, 0.5 0.75, 1, 1.5, 2, 4, 8, 12, 24, 28, 32, 36, 48, 52, 56, 72 and 96 h. Faecal samples were taken at 0, 24, 48, 72, 96 and 168 h for drug analysis, bacteriological examination, SCFA analysis and measurement of faecal dry matter content. There were no faecal samples taken at 72 h after drug administration to ponies.

6.2.2 Intravenous administration of oxytetracycline to ponies with cannulated caecal fistulas

Three pony mares with cannulated caecal fistulas (No. I-III), as outlined in the general Materials and Methods, were used on up to 3 occasions (1-3). Oxytetracycline hydrochloride was administered by intravenous bolus injection at a dose rate of 10 mg/kg bwt. Plasma samples for drug analysis and caecal liquor samples for drug analysis and SCFA analysis were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 28, 32, 48, 52, 56, 72, 96 and 168 h. In pony III1 samples were taken for up to 72 h, in pony III2 samples were taken at 0.5, 1, 2, 4, 7, 24, 31, 48 and 72 h, and in pony III3 an additional sample was taken at 0.083 h and sampling was stopped at 48 h. The pH of caecal liquor was measured in ponies I and II only. Bacteriological examinations of caecal liquor were carried out at 0, 24, 48, 72, 96 and 168 h in ponies I and II, at 0, 24, 48 and 336 h in pony III1, and at 0, 24, 48, 72 and 144 h in pony III2. Faecal samples were taken for drug analysis, SCFA analysis and measurement of dry matter content from ponies I and II at 0, 24, 48, 72, 96 and 168 h. Plasma biochemistry and haematological examinations were carried out at 0, 24, 48, 72, 96 and 168 h in ponies I and II, and at 0, 24, 48, 72 and 144 h in pony III1.

6.2.3 Oral administration of oxytetracycline to ponies with cannulated caecal fistulas

Two pony mares with cannulated caecal fistulas (No. I and II), as outlined in the general Materials and Methods, were used on 3 occasions (1-3) at least 10 months after intravenous administration of oxytetracycline hydrochloride. Oxytetracycline hydrochloride was administered *via* nasogastric tube at a dose rate of 10 mg/kg bwt. On occasions 1 and 2, plasma samples for drug analysis and caecal liquor samples for drug analysis, pH

measurement and SCFA analysis were taken at 0, 0.25, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 28, 32, 48, 52, 56, 72, 96, 168 and 336 h. Bacteriological examinations of caecal liquor were carried out at 0, 24, 48, 72, 96, 168 and 336 h. Faecal samples were taken for the measurement of drug concentrations, SCFA concentrations and dry matter content at 0, 24, 48, 72, 96, 168 and 336 h. Samples were taken for plasma biochemistry and for haematological examinations at 0, 24, 48, 168 and 336 h. On occasion 3, caecal liquor samples were taken at 0, 24, 48, 72, 96 and 168 h for a limited bacteriological examination (coliform and streptococcal counts and the isolation of *Salmonella spp.*) and measurement of pH and SCFA concentrations. Faecal samples were taken at 0, 24, 48, 72, 96 and 168 h for isolation of *Salmonella spp.*, SCFA analysis and the measurement of dry matter content. Samples were taken for plasma biochemistry and haematological examinations at 0, 24, 48 and 168 h.

6.2.4 *In vitro* studies with oxytetracycline

Oxytetracycline hydrochloride was incubated with caecal liquor for 3 and 24 h, and drug concentrations and SCFA concentrations were measured, and bacteriological examinations were carried out, as outlined in the general Materials and Methods. In addition, oxytetracycline hydrochloride concentrations were measured following incubation at pH 1.9 as outlined in the general Materials and Methods.

6.3 Results of intravenous administration of oxytetracycline to horses, ponies and donkeys

6.3.1 Plasma disposition and pharmacokinetics

A semilogarithmic plot of plasma concentrations (mean±SEM) of oxytetracycline, following intravenous administration to horses, ponies and donkeys, is shown in Figure 6-1. Individual plasma concentration versus time data is given in Appendix D (Tables D1-D3). In horses and ponies, the plasma concentration versus time plots were similar and oxytetracycline persisted at measurable concentrations (>0.08 μg/ml) in plasma for 96 h in horses and 72 h in ponies. In donkeys, the slope of the terminal phase of the plasma concentration versus time plot was steeper than in horses and ponies and oxytetracycline could be detected in plasma for 48 h only.

The mean pharmacokinetic parameters calculated from the bi-exponential equations used to describe the plasma concentration versus time data are given in Table 6-1, and the data from individual animals is given in Appendix D (Tables D4-D6). The harmonic mean of the elimination half-lives calculated for horses and ponies was similar, whilst the value calculated for donkeys was *circa* 50% of this. The AUC and AUMC were around twice as

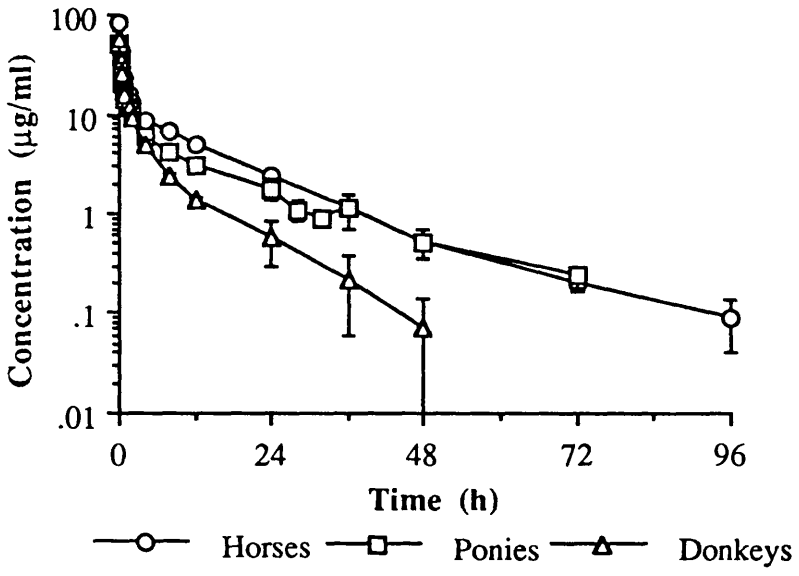

Figure 6-1. Plasma concentrations (mean±SEM) of oxytetracycline following intravenous administration to horses, ponies and donkeys

Parameter	Horses (n=3)	Ponies (n=4)	Donkeys (n=3)
t1/2 B2 (h)*	0.15	0.23	0.29
t1/2 B1 (h)*	11.69	11.77	5.40
Cp0 (µg/ml)	111.18±17.99	59.51±13.86	67.10±4.22
Vc (ml/kg)	94.34±13.67	204.50±56.41	150.20±9.30
AUC_{obs} (µg.h/ml)	217.17±7.56	156.23±29.70	97.32±4.29
$AUMC_{obs}$ (µg.h^2/ml)	3045.47±152.14	2399.18±608.06	631.53±183.17
AUC (µg.h/ml)	253.53±4.72	182.35±35.11	109.45±0.75
AUMC (µg.h^2/ml)	3949.47±269.97	2898.98±699.09	780.41±228.67
MRT (h)*	14.05±0.78	15.10±1.36	6.67±2.23
Vd_{area} (ml/kg)	672.79±49.74	1048.23±218.47	776.52±193.43
Vd_{ss} (ml/kg)	615.97±47.36	956.26±202.98	649.27±185.17
CL_b (ml/h.kg)	39.31±0.59	60.77±10.76	91.37±0.63
k_{el} (/h)	0.44±0.08	0.32±0.03	0.59±0.08
k_{21} (/h)	0.60±0.02	0.54±0.02	0.61±0.04
k_{12} (/h)	3.52±0.83	2.16±0.33	1.92±0.72

Table 6-1. Disposition kinetics of oxytetracycline in plasma following intravenous administration to horses, ponies and donkeys

Key: data as mean±SEM; * harmonic mean

high in horses and ponies compared with donkeys. There was a statistically significant difference in the variance of the AUC ($p = 0.043$) and the AUMC ($p = 0.043$), calculated using a Kruskal Wallis test. However, there were no statistically significant differences detected between the groups using a Mann Whitney U test. The CL_b was lowest in the horses, *circa* twice this in the ponies, and highest in donkeys (*circa* three times the horse value). There was a statistically significant difference ($p = 0.043$) in the CL_b of horses, ponies and donkeys calculated using a Kruskal Wallis test, but there were no statistically significant differences detected using a Mann Whitney U test.

The plasma concentration versus time data from pony 10 and donkey 16 were best described using a tri-exponential equation, and the pharmacokinetic parameters calculated from these equations are given in Table 6-2.

6.3.2 Faecal concentrations

Oxytetracycline concentrations (mean±SEM) in faecal samples following intravenous administration to horses, ponies and donkeys are shown in Figure 6-2, and the data from individual animals is given in Appendix D (Tables D7-D9). Faecal drug concentrations were measured for up to 96 h in all 3 groups. Faecal oxytetracycline concentrations were higher than the simultaneous plasma concentrations in all the animals at all of the sampling times.

6.3.3 Bacteriological examinations of faeces

Counts of viable bacteria in faeces following intravenous administration of oxytetracycline are shown in Figures 6-3, 6-4 and 6-5, and the data from individual animals is given in Appendix D (Tables D10-D12).

Selective isolation of *Salmonella spp.* was not carried out. *Clostridium difficile* was looked for but not isolated.

In the horses, there were no marked changes in the number of coliforms or *Bacteroides spp.* isolated from faeces following intravenous administration of oxytetracycline (Figure 6-3). There were moderately high numbers of coliforms isolated from horse 3 (10^8/g) at 72 h after drug administration. There was an increase in the mean number of streptococci (to 10^9/g) isolated at 96 h after drug administration. There were high numbers of streptococci isolated from horse 3 at 48, 72, 96 and 168 h (10^8-10^{10}/g). The mean number of viable lactobacilli increased to 10^9-10^{11}/g at 48 and 96 h after drug administration. This was the result of an increase in the number of viable lactobacilli isolated from horse 1 (10^9-10^{11}/g) at 48, 96 and 168 h and from horse 3 (10^{10}/g) at 96 h after drug administration. The number of *Bacteroides spp.* isolated from horse faeces was low (10^4-10^8/g) throughout the study.

Parameter	Pony 10	Donkey 16
t1/2 B3 (h)	0.18	0.12
t1/2 B2 (h)	1.29	1.64
t1/2 B1 (h)	12.18	14.66
Cp0 (μg/ml)	60.7	71.09
Vc (ml/kg)	164.75	140.66
AUC_{obs} (μg.h/ml)	141.35	89.92
$AUMC_{obs}$ (μg.h^2/ml)	1633.55	997.87
AUC (μg.h/ml)	137.71	89.75
AUMC (μg.h^2/ml)	1795.11	1211.80
MRT (h)	11.56	11.10
Vd_{area} (ml/kg)	1275.87	2356.81
Vd_{ss} (ml/kg)	946.57	1504.40
CL_b (ml/h.kg)	72.62	111.42
k_{el} (/h)	0.44	0.75
k_{21} (/h)	1.65	1.36
k_{12} (/h)	1.65	3.12
k_{31} (/h)	0.17	0.10
k_{13} (/h)	0.62	0.83

Table 6-2. Disposition kinetics of oxytetracycline in plasma following intravenous administration to pony 10 and donkey 16

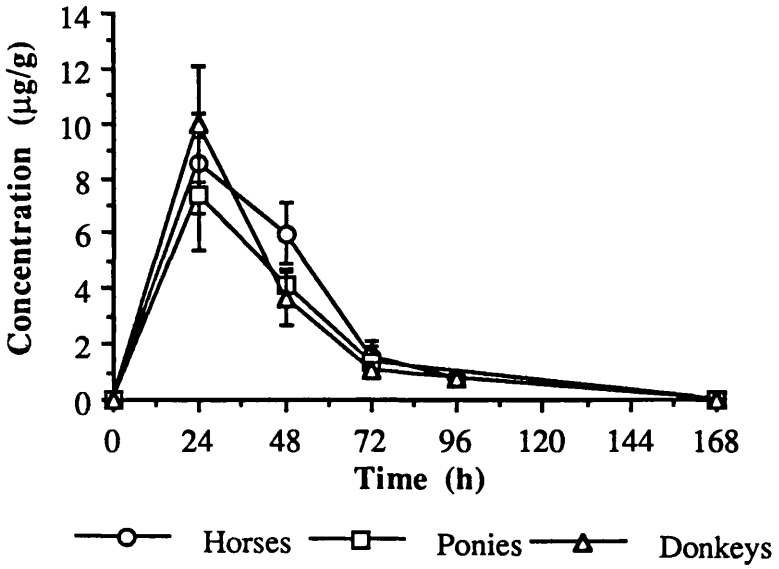

Figure 6-2. Faecal concentrations (mean±SEM) of oxytetracycline following intravenous administration to horses, ponies and donkeys

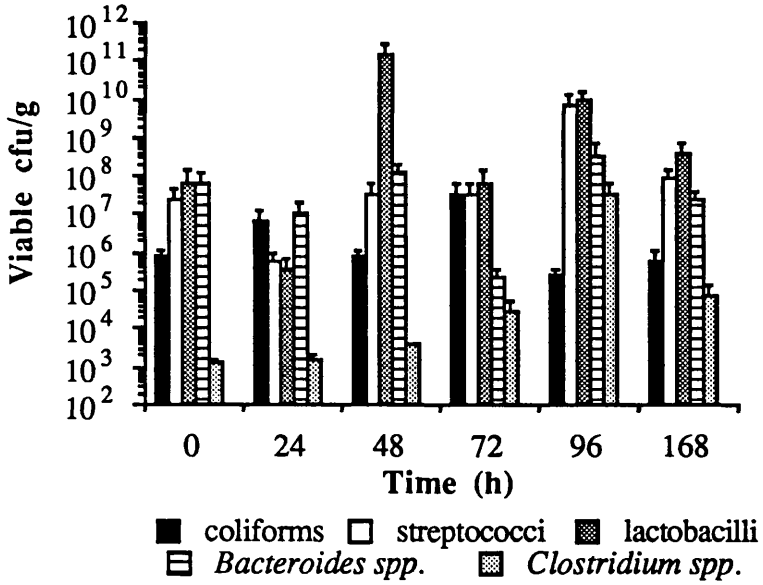

Figure 6-3. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of oxytetracycline to horses

There was an increase in the mean number of viable *Clostridium spp.* (to 10^7/g) isolated at 96 h after drug administration due to an increase in the number of these organisms (to 10^6-10^8/g) isolated from the faeces of horses 1 and 2.

There were no marked alterations in the mean number of bacteria isolated from the ponies at any of the sampling times, except for an increase in the mean number of viable streptococci (up to 10^9/g) isolated at 24 h after drug administration (Figure 6-4). There were high numbers of streptococci (10^8-10^{10}/g) isolated from pony 8 at 24, 48, 72 and 168 h after oxytetracycline administration. The mean number of *Bacteroides spp.* isolated was low (<10^9/g) prior to and at 72 h after drug administration. In addition, there were moderately high numbers of *Clostridium spp.* (10^6/g) isolated at 24 and 48 h from pony 7, and at 48 and 72 h from pony 9.

There was an increase in the mean number of coliforms (up to 10^8/g) isolated from donkey faeces at 72 and 96 h after oxytetracycline administration (Figure 6-5). There was an increase in the number of coliforms isolated from donkey 17 (to 10^9/g) at 72 h, and from donkey 16 (to 10^9/g) at 96 h. There was an increase in the mean number of lactobacilli (up to 10^8/g) isolated at 72 h after drug administration, which was primarily due to an increase in the number isolated from donkey 17 at this time. The number of *Bacteroides spp.* isolated from donkey faeces were low at all times, except at 96 h after drug administration. There were no alterations in the number of streptococci or *Clostridium spp.* isolated from donkey faeces following a single intravenous administration of oxytetracycline.

6.3.4 Faecal SCFA concentrations

Faecal lactic acid and total VFA concentrations (mean±SEM) following intravenous administration of oxytetracycline to horses, ponies and donkeys are shown in Figures 6-6 and 6-7, and the mean data and data from individual animals are given in Appendix D (Tables D13-D23). Lactic acid concentrations (mean±SEM) were increased to 25.1±17.0 mmol/kg at 48 h in horses, and to 6.4±3.3 mmol/kg at 48 and 72 h in ponies, although they were only outside the normal range of 0.0-24.4 mmol/l in horses (Figure 6-6). The increase in the mean lactic acid concentration in horses was due to an increased lactic acid concentration in the faecal samples from horses 2 and 3 to 17.7 and 57.5 mmol/kg, respectively, although the 48 h sample from horse 3 was the only sample outside the normal range. In ponies, there was an increase in lactic acid concentrations at 48 h in pony 7 and at 72 h in pony 8 to 14.3 and 36.5 mmol/kg, respectively, but only the sample from pony 8 was outside the normal range. Lactic acid concentrations in donkey faecal samples were all within the normal range and showed no increases following intravenous administration of oxytetracycline.

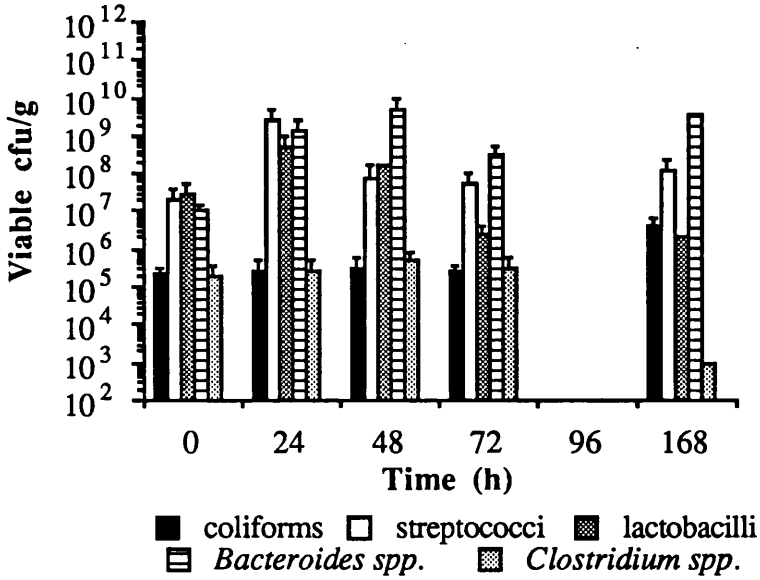

Figure 6-4. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of oxytetracycline to ponies

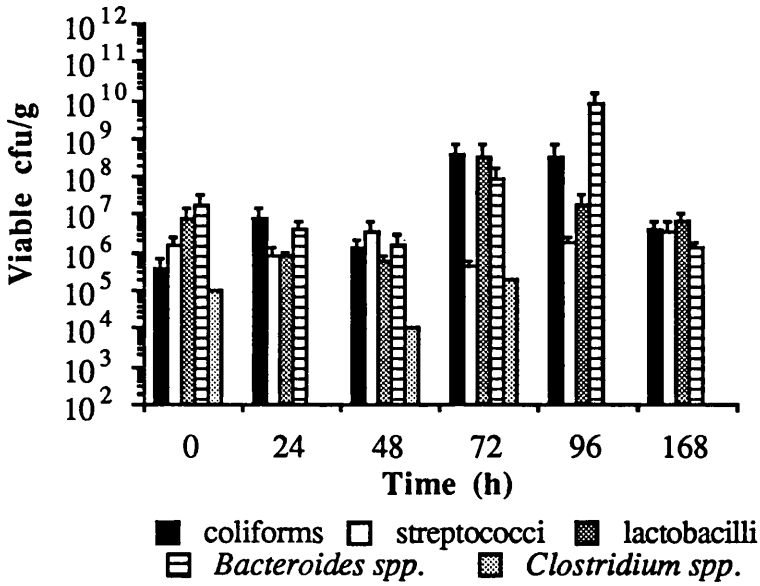

Figure 6-5. Counts of viable bacteria (mean±SEM) in faeces following intravenous administration of oxytetracycline to donkeys

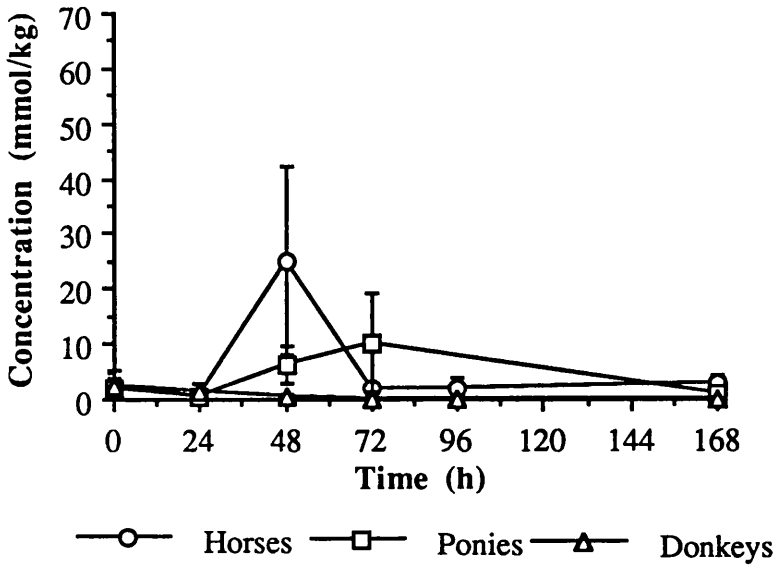

Figure 6-6. Lactic acid concentrations (mean±SEM) in faeces following intravenous administration of oxytetracycline to horses, ponies and donkeys

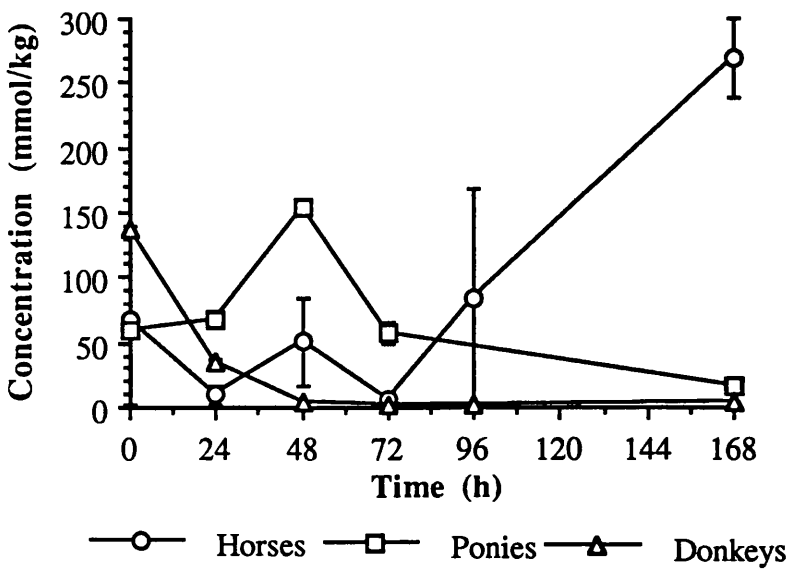

Figure 6-7. Total VFA concentrations (mean±SEM) in faeces following intravenous administration of oxytetracycline to horses, ponies and donkeys.

The total VFA concentrations (mean±SEM) in faeces from horses, ponies and donkeys following intravenous administration of oxytetracycline were very variable (Figure 6-7). In addition, there was considerable variation in individual VFA concentrations. In horses, total VFA concentrations were lower than the normal range of 24.4-109.2 mmol/l at 24 and 72 h and higher than normal at 168 h after intravenous administration of oxytetracycline. In ponies, mean total VFA concentrations were higher than the normal range at 48 h and lower than normal at 168 h. In donkeys, the mean total VFA concentration was elevated above the normal range prior to and below normal at 48, 72, 96 and 168 h after intravenous administration of oxytetracycline. There were no trends in VFA concentrations in faeces that were associated with the intravenous administration of oxytetracycline.

6.3.5 Faecal dry matter content and consistency

The faecal dry matter content (mean±SEM) following intravenous administration of oxytetracycline to horses, ponies and donkeys is shown in Figure 6-8. The mean values and data from individual animals are given in Appendix D (Tables D24-D26). The mean faecal dry matter content were reduced to <17% in horses at 96 h after oxytetracycline administration. There was a reduction in the faecal dry matter content at 24, 48, 72 and 96 h in horse 1 (14.40-16.50%) , at 48 h in horse 2 (15.20%), and at 96 h in horse 3 (16.00%). In ponies, the mean faecal dry matter content was reduced to <17% at 48 h after drug administration. There was a reduction in the faecal dry matter content to 13.00-16.40% in pony 7 at 48, 72 and 168 h. The faecal dry matter content was low (11.20-15.60%) in pony 8 at 0, 24, 48 and 72 h after drug administration. The faecal dry matter content was low (12.50%) at 0 h in pony 10. The mean faecal dry matter content was reduced to 13.93-16.90% in donkeys at 24, 48, 72 and 96 h after intravenous administration of oxytetracycline. Faecal dry matter content was reduced at 48, 72 and 96 h in donkeys 15 and 16 (10.00-16.70%), and at 0, 24 and 48 h in donkey 17 (13.30-15.30%).

The faecal consistency was soft at 48, 72 and 96 h and as slightly soft at 24 h after drug administration to all 3 horses. The faecal consistency was soft in ponies 8 and 10 prior to drug administration. The faecal consistency was soft in donkey 17 prior to and at 24 h, in donkey 16 at 48 h, in all 3 donkeys at 72 h, and in donkey 16 at 96 h after drug administration. In addition, donkey 15 was noted to have passed a reduced volume of faeces at 48 h after drug administration.

6.3.6 Bromsulphalein clearance in horses and donkeys

A plot of the plasma bromsulphalein concentration versus time is shown in Figure 6-9. The elimination half-life of bromsulphalein was 2.5 min for each of the 3 horses (No. 1-3) and

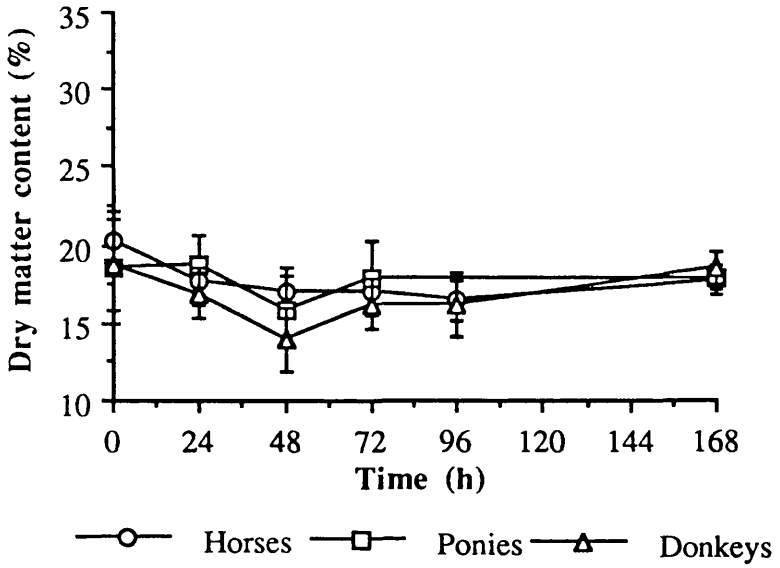

Figure 6-8. Faecal dry matter content (mean±SEM) following intravenous administration of oxytetracycline to horses, ponies and donkeys

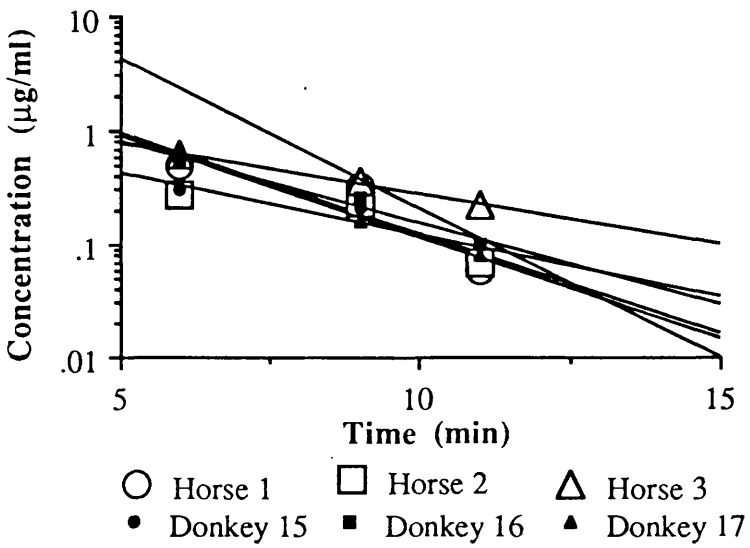

Figure 6-9. Plasma concentrations of bromsulphalein following intravenous administration to horses and donkeys

1.3, 3.3 and 1.8 min for the 3 donkeys (No. 15-17, respectively) indicating similar liver clearance of the compound.

6.4 Results of intravenous administration of oxytetracycline to ponies with cannulated caecal fistulas

6.4.1 Plasma disposition and pharmacokinetics

The plasma concentrations of oxytetracycline following intravenous administration to ponies I-III on occasions 1-3 are given in Appendix D (Table D27).

A bi-exponential equation best described the plasma concentration versus time data from ponies I1, I2, III1 and III2. In addition, a tri-exponential equation was used to describe the data from ponies II1, II2 and III3. The pharmacokinetic parameters calculated from the plasma concentration versus time data from each animal on each occasion are given in Tables 6-3 and 6-4. The pharmacokinetic parameters that were calculated were similar to those obtained from ponies 7-10, except for pony III1 which had a very long elimination half-life.

6.4.2 Caecal liquor and faecal concentrations

The caecal liquor concentrations of oxytetracycline following intravenous administration to ponies I-III on occasions 1-3 are shown in Figures 6-10 and 6-11, and the individual data is given in Appendix D (Table D28). Oxytetracycline was measured in the caecal liquor for up to 72 h after a single intravenous administration. The maximum caecal liquor concentrations were 1.36, 1.37. 1.23, 1.52, 1.82 1.85 and 2.41 μg/ml in ponies, I1, I2, II1, II2, III1, III2 and III3, respectively. The time taken for oxytetracycline to reach the caecum varied from animal to animal. A maximum concentration of oxytetracycline was measured at 4 h in ponies I1, I2 and III3, at 28 h pony II1, at 12 h in ponies II2 and III1, and at 7 h in pony III2.

Drug disposition within the caecum was described using the AUC and the AUMC for observed values and the ratio of these (MRT). The pharmacokinetic variables that were calculated are given in Table 6-5.

Faecal concentrations of oxytetracycline following intravenous administration to ponies I and II are shown in Figure 6-12, and the individual data is given in Appendix D (Table D29). Oxytetracycline was measured in faecal samples for up to 72 h following a single intravenous administration.

Parameter	I1	I2	II1	II2	III1	III2	III3
t1/2 B2 (h)	0.31	0.35	0.44	0.37	0.07	0.36	0.42
t1/2 B1 (h)	10.48	12.08	12.07	11.53	17.48	10.96	9.45
Cp0 (μg/ml)	42.17	51.33	27.22	33.04	101.02	70.63	52.60
Vc (ml/kg)	237.11	194.82	367.44	302.64	98.99	141.58	121.07
AUC_{obs} (μg.h/ml)	106.14	200.57	82.23	142.96	210.15	231.32	154.18
$AUMC_{obs}$ (μg.h^2/ml)	1299.18	3038.85	1106.14	2116.66	4695.10	2896.84	1347.25
AUC (μg.h/ml)	113.26	189.76	88.65	157.70	196.20	228.05	157.41
AUMC (μg.h^2/ml)	1480.10	3295.31	1298.06	2416.64	4697.15	3149.77	1553.53
MRT (h)	12.24	15.15	13.45	14.81	22.34	12.52	8.74
Vd_{area} (ml/kg)	1334.89	833.99	1965.01	1054.94	1285.52	693.56	866.41
Vd_{ss} (ml/kg)	1153.80	754.90	1651.90	971.71	1220.18	605.66	626.98
CL_b (ml/h.kg)	88.29	47.86	112.81	63.41	50.97	43.85	63.53
k_{el} (/h)	0.37	0.25	0.31	0.21	0.52	0.31	0.53
k_{21} (/h)	0.40	0.46	0.29	0.54	0.72	0.40	0.23
k_{12} (/h)	1.55	1.33	1.02	1.20	8.16	1.30	0.95

Table 6-3. Disposition kinetics of oxytetracycline in plasma following intravenous administration to ponies I1, I2, II1, II2, III1, III2 and III3

Parameter	II1	II2	III3
t1/2 B3 (h)	0.19	0.21	0.27
t1/2 B2 (h)	1.81	1.79	0.56
t1/2 B1 (h)	14.94	13.40	10.24
Cp0 (μg/ml)	39.65	36.38	80.90
Vc (ml/kg)	252.22	274.90	123.61
AUC_{obs} (μg.h/ml)	83.78	143.38	154.49
$AUMC_{obs}$ (μg.h^2/ml)	1106.53	2116.77	1365.73
AUC (μg.h/ml)	83.07	143.84	151.69
AUMC (μg.h^2/ml)	1224.30	2255.25	1513.11
MRT (h)	13.21	14.76	8.84
Vd_{area} (ml/kg)	2595.14	1344.45	973.82
Vd_{ss} (ml/kg)	1774.18	1090.04	657.62
CL_b (ml/h.kg)	120.38	69.52	65.93
k_{el} (/h)	0.48	0.25	0.53
k_{21} (/h)	1.22	1.53	2.20
k_{12} (/h)	1.79	1.42	0.16
k_{31} (/h)	0.11	0.17	0.18
k_{13} (/h)	0.52	0.35	0.77

Table 6-4. Disposition kinetics of oxytetracycline in plasma following intravenous administration to ponies II1, II2 and III3

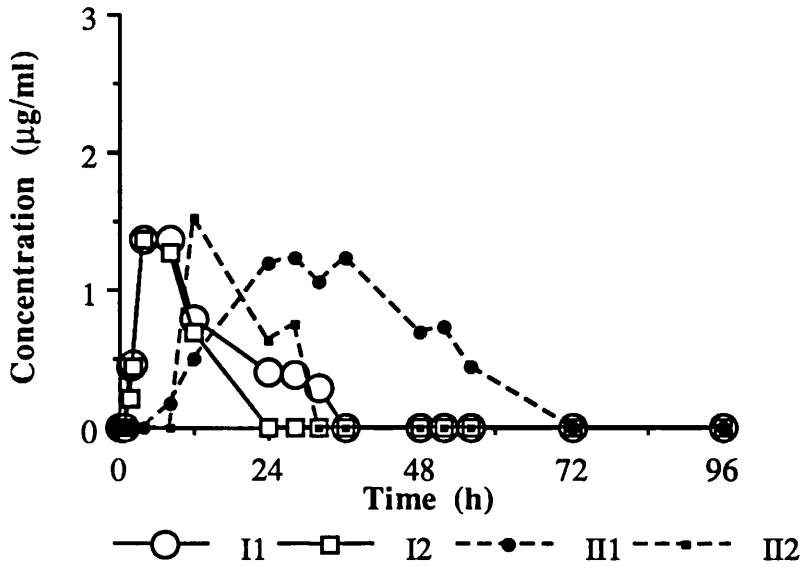

Figure 6-10. Caecal liquor concentrations of oxytetracycline following intravenous administration to ponies I and II

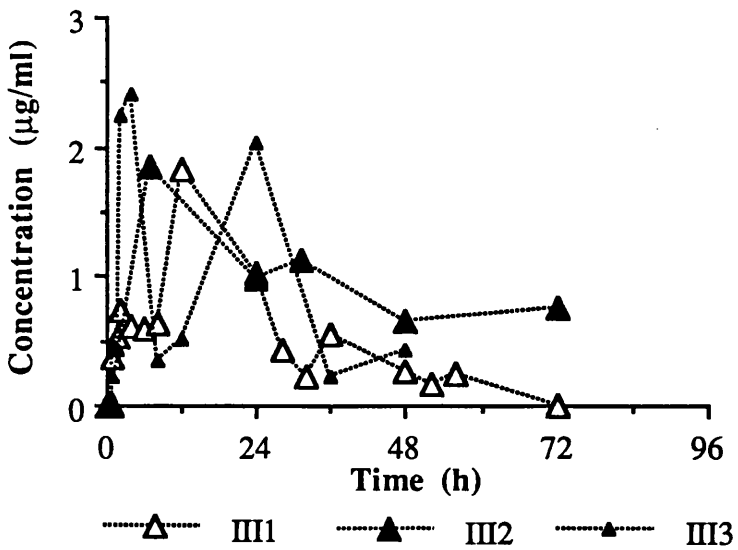

Figure 6-11. Caecal liquor concentrations of oxytetracycline following intravenous administration to pony III

Parameter	I1	I2	II1	II2	III1	III2	III3
AUC_{obs} (μg.h/ml)	22.43	15.42	46.14	20.28	40.65	69.71	45.72
$AUMC_{obs}$ (μg.h^2/ml)	292.68	125.25	1579.20	352.80	909.40	2165.76	905.95
MRT (h)	13.05	8.12	34.23	17.40	22.37	31.07	19.82

Table 6-5. Disposition kinetics of oxytetracycline in caecal liquor following intravenous administration to ponies

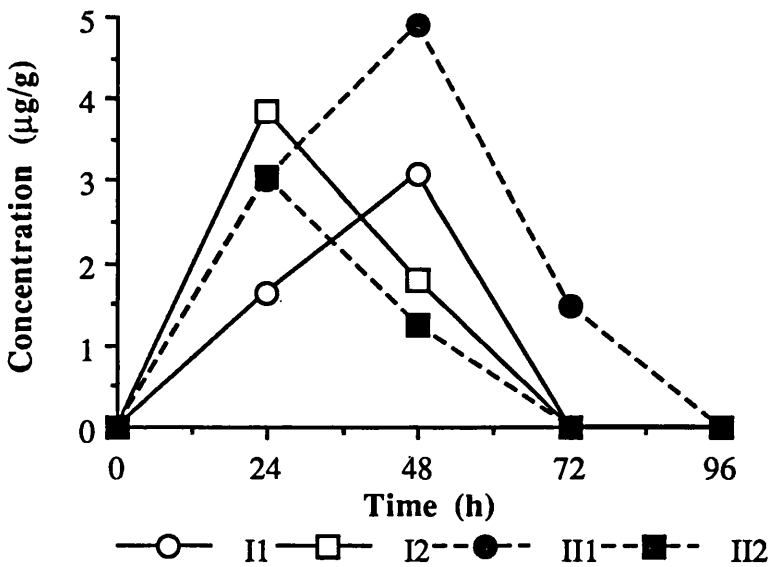

Figure 6-12. Faecal concentrations of oxytetracycline following intravenous administration to ponies

6.4.3 Bacteriological examinations

Counts of viable bacteria in caecal liquor following intravenous administration of oxytetracycline are shown in Figures 6-13 to 6-17, and the individual data is given in Appendix D (Table D30).

Salmonella spp. and *C. difficile* were selected for but not isolated.

There were no marked changes in the number of coliforms isolated from ponies I and II on occasions 1 and 2, although there was a slight increase (to 10^8/ml) at 48 h in pony I1 (Figure 6-13). Pony III1 had a high coliform count of 10^8-10^{10}/ml at 24, 48 and 336 h after drug administration, and in pony III2 the coliform counts were high (10^8-10^{10}/ml) at 0, 48, 72 and 144 h after drug administration.

The number of streptococci isolated from ponies I1, I2 and II2 did not alter markedly following the intravenous administration of oxytetracycline (Figure 6-14). In pony II1, there was an increase in the number of streptococci (10^8/ml) isolated at 24 h after drug administration. The number of streptococci isolated from pony III was high (10^8-10^{10}/ml) prior to drug administration on occasions 1 and 2, at 24, 48 and 336 h after drug administration on occasion 1, and at 24, 72 and 144 h on occasion 2. *Streptococcus intermedius* was identified using the API system.

There were no marked alterations in the number of lactobacilli isolated from ponies I and II on occasions 1 and 2 (Figure 6-15). In pony III the number of lactobacilli isolated was increased to 10^8-10^{10}/ml at 24 and 48 h on occasion 1, and prior to and at 144 h after drug administration on occasion 2. *Lactobacillus acidophilus* was identified using the API system.

The number of *Bacteroides spp.* isolated prior to drug administration was low (<10^9/ml) in ponies I1, II2, III1 and III2 (Figure 6-16). There were low numbers (<10^9/ml) of *Bacteroides spp.* isolated from pony I1 at 24, 48, 72 and 168 h, from pony I2 at 24, 48, 72, 96 and 168 h, from pony II1 at 24, 72 and 96 h, from pony II2 at 24, 48, 72 and 96 h, and from ponies III1 and III2 at 24 and 48 h after intravenous administration of oxytetracycline. *Bacteroides spp.* identified using the API system were *B. oralis*, *B. ovatus*, *B. thetaiotaomicron* and *B. uniformis*.

There were no marked alterations in the number of *Clostridium spp.* isolated from any of the ponies at any of the sampling times prior to or following the intravenous administration of oxytetracycline, although there were moderately high numbers (10^6/ml) isolated from pony I2 prior to drug administration (Figure 6-17). *Clostridium spp.* identified using the API

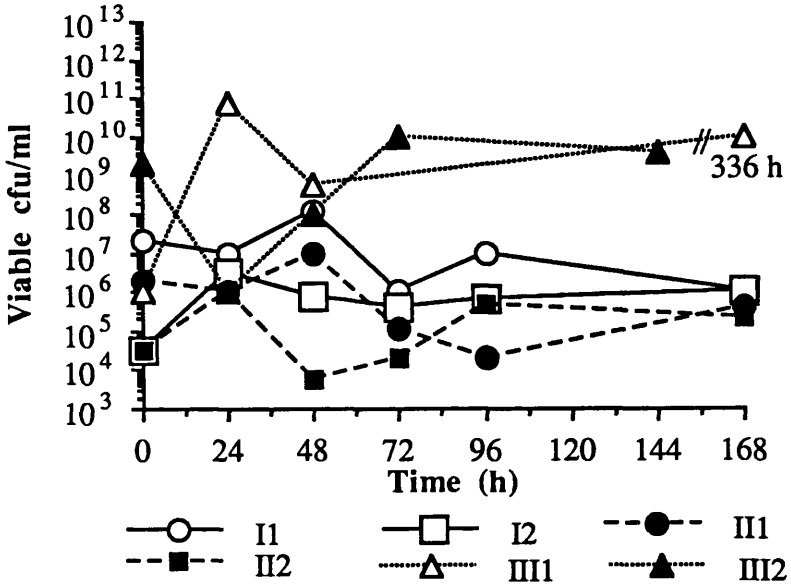

Figure 6-13. Counts of viable coliforms in caecal liquor following intravenous administration of oxytetracycline to ponies

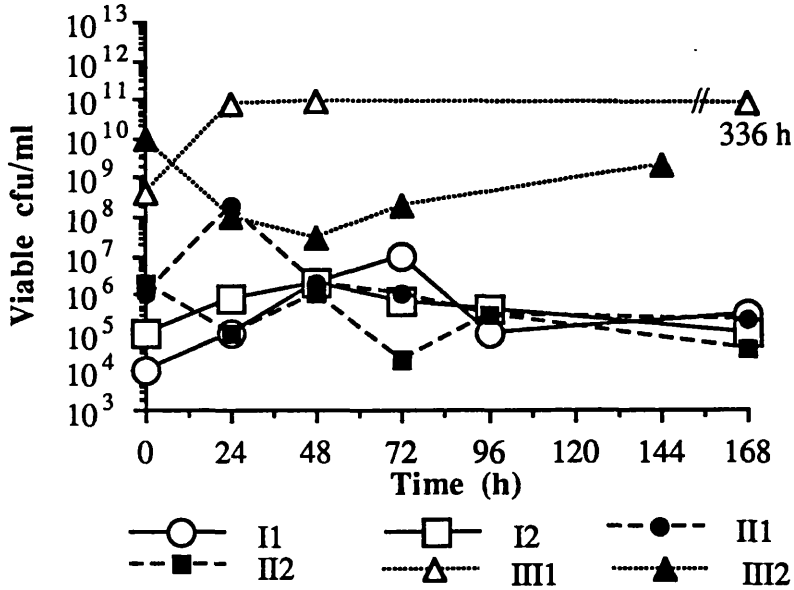

Figure 6-14. Counts of viable streptococci in caecal liquor following intravenous administration of oxytetracycline to ponies

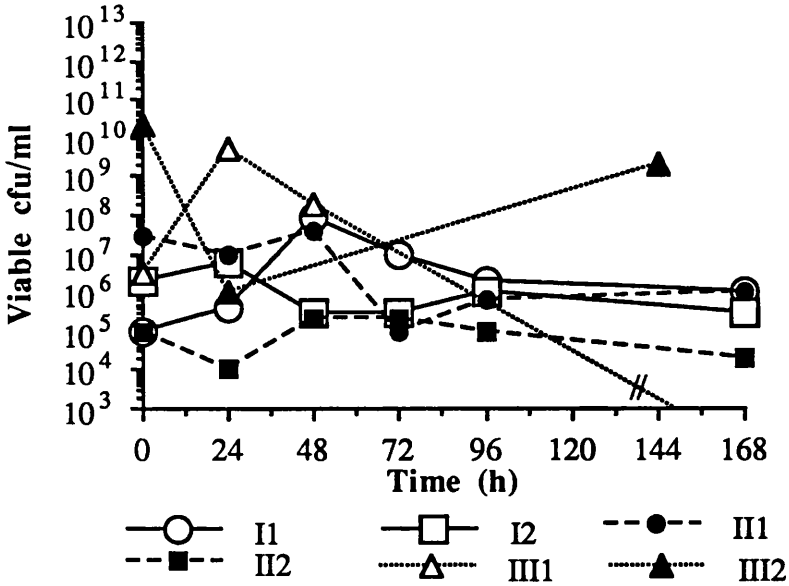

Figure 6-15. Counts of viable lactobacilli in caecal liquor following intravenous administration of oxytetracycline to ponies

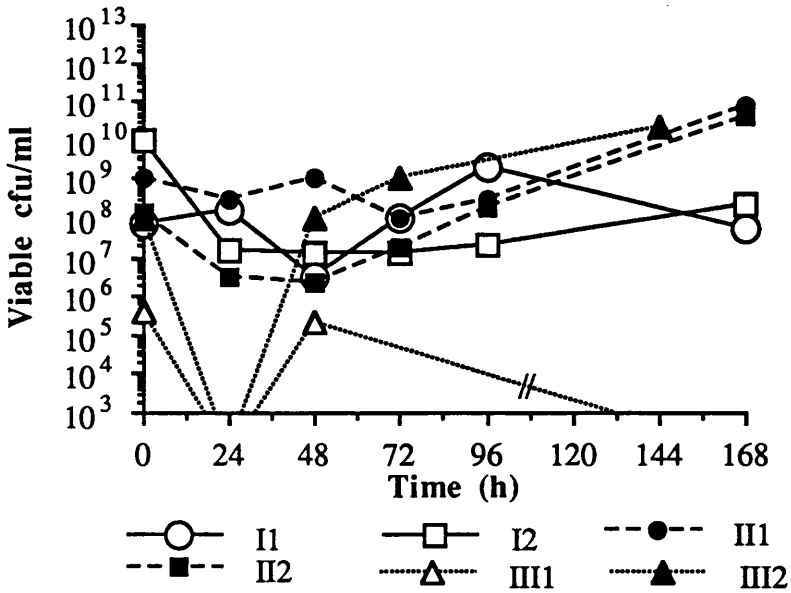

Figure 6-16. Counts of viable *Bacteroides spp.* in caecal liquor following intravenous administration of oxytetracycline to ponies

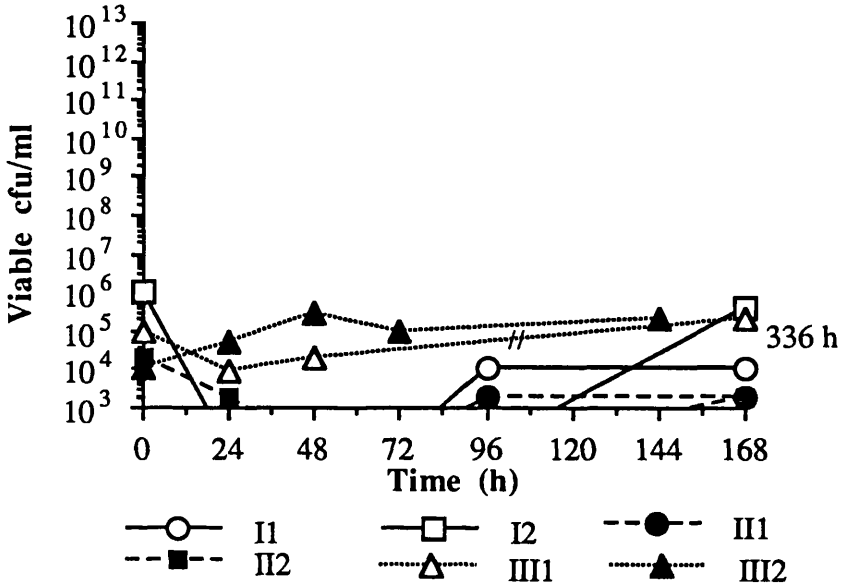

Figure 6-17. Counts of viable *Clostridium spp.* in caecal liquor following intravenous administration of oxytetracycline to ponies

system were *C. butyricum*, *C. perfringens* (10^3/ml from pony I2 at 168 h after drug administration) and *C. ramosum.*

Other bacteria identified using the API system were *Actinomyces israelii*, *Eubacterium lentum*, *Peptococcus asaccharolyticus* and *Veillonella parvula.*

6.4.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

Caecal liquor pH values following intravenous administration of oxytetracycline are shown in Figure 6-18, and the individual data is given in Appendix D (Table D31). The caecal liquor pH was high (>7.2) prior to and at 2, 4, 8, 12 and 52 h after drug administration and low (<6.8) at 168 h after drug administration to pony I1. In pony I2, caecal liquor pH was high at 1.5 h and low at 8, 52, 72 and 96 h after drug administration. Caecal liquor pH was high in pony II1 at 0, 8, 12, 24, 72, 96 and 168 h and in pony II2 at 0.5, 0.75, 24, 72, 96 and 168 h after intravenous administration of oxytetracycline.

Lactic acid concentrations in caecal liquor, following intravenous administration of oxytetracycline to ponies, are shown in Figure 6-19, and the individual data is given in Appendix D (Tables D32a-D38a). Lactic acid concentrations were increased to 5.4-20.3 mmol/l in pony I1 at 4, 8, 24, 28, 32, 36, 48, 52, 56 and 72 h, in pony I2 at 1, 1.5, 4, 8, 12, 52 and 72 h, in pony III1 at 4, 8, 12, 24, 28, 32, 36, 52, 56 and 72 h in pony III1, and in pony III2 at 7, 31 and 72 h but all the concentrations lay within the normal range of 0.0-24.4 mmol/l. There were no increases in caecal liquor lactic acid concentrations in pony II2. In pony II1, caecal lactic acid concentrations were increased to 6.9-40.2 mmol/l at 28, 32, 36, 48, 52, 56 and 72 h after drug administration, and were higher than the established normal range at 48, 52, 56 and 72 h. In pony III3, caecal liquor lactic acid concentrations were elevated to 7.5-32.6 mmol/l at 0.75, 1. 1.5, 4, 8, 12 and 36 h after drug administration, and were above the normal range at 4 and 8 h.

Total VFA concentrations in caecal liquor are shown in Figure 6-20, and the total VFA and individual acid concentrations are given in Appendix D (Tables D32a-D38a). Total and individual VFA concentrations fluctuated around the normal ranges. Total VFA concentrations in caecal liquor were increased to 137.7-143.3 mmol/l, compared with the normal range of 24.4-109.2 mmol/l, at 1.5 and 8 h in pony I2. There was a reduction in the total VFA concentrations to 18.3-19.4 mmol/l at 1 and 72 h in pony III1. There were some alterations in the individual VFA concentrations following intravenous administration of oxytetracycline. There was a reduction in acetic acid concentrations to 7.2-9.4 mmol/l, compared with the normal range of 12.6-64.5 mmol/l, at 0.75 h in pony II2 and at 8 h in

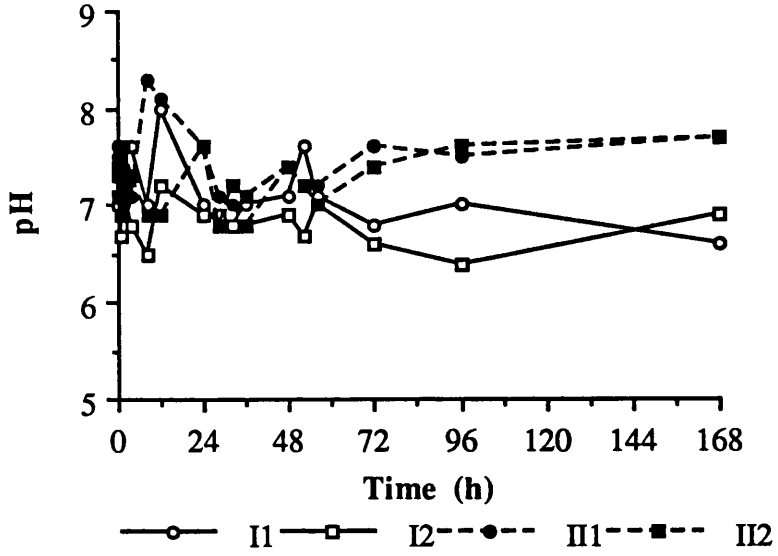

Figure 6-18. Caecal pH following intravenous administration of oxytetracycline to ponies

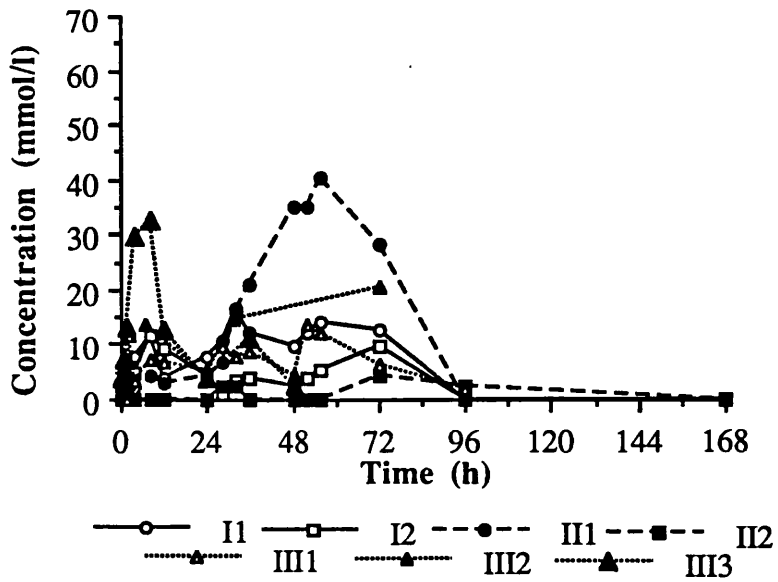

Figure 6-19. Lactic acid concentrations in caecal liquor following intravenous administration of oxytetracycline to ponies

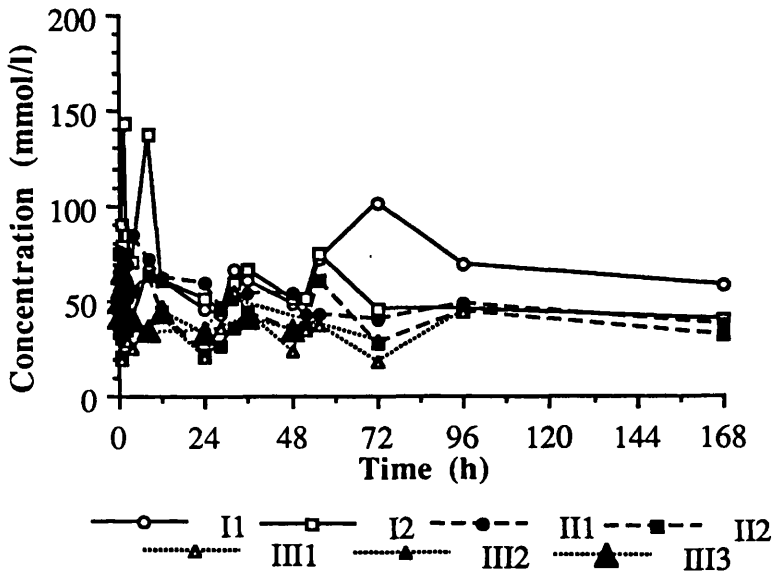

Figure 6-20. Total VFA concentrations in caecal liquor following intravenous administration of oxytetracycline to ponies

pony III1. There was a reduction in the propionic acid concentrations to 1.6-4.0 mmol/l, compared with the normal range of 4.7-24.5 mmol/l, at 0.75, 12, 24, 48, 52 and 56 h in pony I1, at 8, 12, 28, 32 and 36 h in pony I2, at 0.75, 1 and 28 h in pony II2, and at 6, 24, 48 and 72 h in pony III1. There was an increase in the butyric acid concentrations to 79.1-121.4 mmol/l, compared with the normal range of 4.8-67.3 mmol/l, at 1.5 and 8 h in pony I2. Butyric acid concentrations were reduced to 0.8-3.8 mmol/l at 1, 8, 12, 24, 28, 52, 72 and 144 h after drug administration to pony III1, and at 8 h in pony III3.

The proportions of acetic, propionic and butyric acid concentrations, as a percentage of the total VFA concentrations, are given in Appendix D (Tables D32b-38b). The ratio of acetic acid was reduced to 10.0%, compared with the normal range of 27.9-67.0%, at 8 h in pony I2. There was an increase in the percentage of acetic acid to 76.6-73.7% at 52 and 144 h in pony III1. The percentage of propionic acid fell to 1.8-3.5%, compared with the normal range of 4.9-38.5%, at 0.75, 12, and 48 h in pony I1, at 8 h in pony I2. There was an increase in the percentage of propionic acid to 48.5-66.0% at 8, 12, 28 and 32 h in pony III1. The ratio of butyric acid was increased to 72.0-88.2%, compared with the normal range of 8.7-66.7%, at 12 h in pony I1 and at 8 h in pony I2. Whereas, the ratio of butyric acid fell to 4.1-6.9% at 1, 28, 52 and 144 h in pony III1. There was an increase in the ratio of propionic plus butyric acids to 78.3-90.0%, compared with the normal range of 27.1-72.1%, at 8 h in ponies I2 and III1.

Faecal SCFA concentrations in ponies following intravenous administration of oxytetracycline are shown in Figures 6-21 and 6-22, and the individual data is given in Appendix D (Tables D39-D42). There were no marked alterations in the lactic acid concentrations in faeces, except there was an increase to 25.7 mmol/kg at 48 h in pony II1, and to 9.0 mmol/kg at 24 h in pony II2, compared with the normal range of 0.0-24.4 mmol/l. There was considerable variation in the faecal VFA concentrations. The total VFA concentrations were lower than the normal range of 24.4-109.2 mmol/l in pony I1. In pony I2, the total VFA concentration was higher than normal at 48, 72 and 96 h after drug administration. In pony II1, there was an elevation of the total VFA concentration at 48 h and a reduction at 72 and 168 h. In pony II2, the total faecal VFA concentration was higher than normal at 0, 24, 48, 72 and 96 h after drug administration, due to a higher than normal (normal 4.8-67.3 mmol/l) butyric acid concentrations at these times. There were no trends in total and individual VFA concentrations that were associated with the intravenous administration of oxytetracycline.

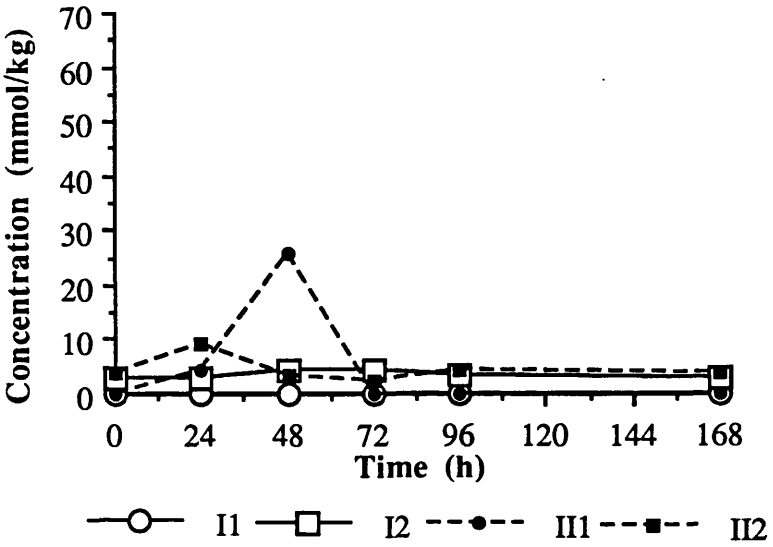

Figure 6-21. Lactic acid concentrations in faeces following intravenous administration of oxytetracycline to ponies

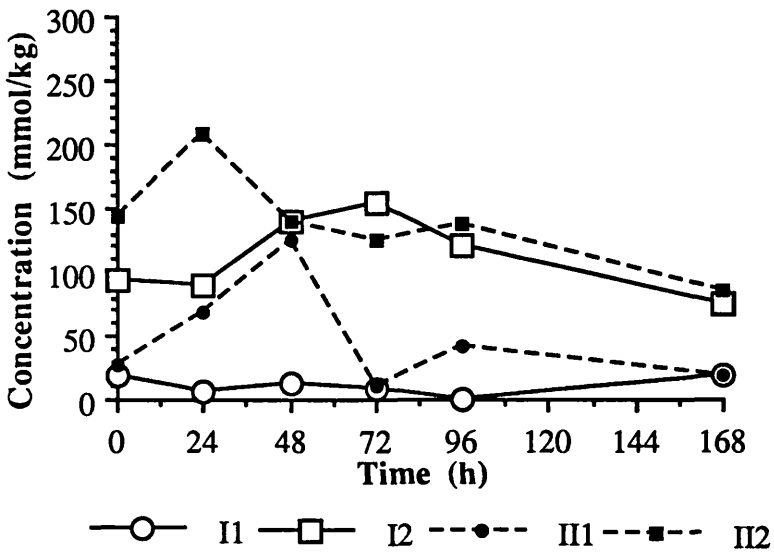

Figure 6-22. Total VFA concentrations in faeces following intravenous administration of oxytetracycline to ponies

6.4.5 Faecal dry matter content and consistency

Faecal dry matter content following intravenous administration of oxytetracycline to ponies is shown in Figure 6-23, and the individual data is given in Appendix D (Table D43). There were few changes in faecal dry matter content. There were no changes observed in faecal consistency.

6.4.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix D (Tables D44-D48) and the results of the haematological examinations are given in Appendix D (Tables D49-D53). Values fluctuated around the normal ranges. There were no alterations in plasma biochemistry or haematology values that were associated with the intravenous administration of oxytetracycline.

6.5 Results of oral administration of oxytetracycline to ponies with cannulated caecal fistulas

Both ponies were depressed in appearance and reluctant to eat at 24, 48, 72 and 96 h after drug administration. This occurred each time the drug was administered, although it was most obvious on the first 2 occasions. In addition, the depression and anorexia were most marked in pony I.

6.5.1 Plasma disposition and pharmacokinetics

The plasma concentrations of oxytetracycline following the oral administration to ponies are shown in Figure 6-24, and the individual data is given in Appendix D (Table D54). The maximum plasma concentrations of 0.53, 0.84, 0.49 and 0.38 μg/ml were measured at 0.75, 1.5, 0.75 and 0.5 h in ponies I1, I2, II1 and II1, respectively.

The pharmacokinetic parameters calculated following oral administration of oxytetracycline to ponies are given in Table 6-6. The MAT was calculated as the difference between the mean MRT following intravenous administration, and the MRT following oral administration for each animal on each occasion. The MAT was very long in all the animals. The bioavailability was calculated using the mean AUC following intravenous administration to each animal and was low.

6.5.2 Caecal liquor and faecal drug concentrations

The caecal liquor concentrations following oral administration of oxytetracycline to ponies are shown in Figure 6-25, and the individual data is given in Appendix D (Table D55). The

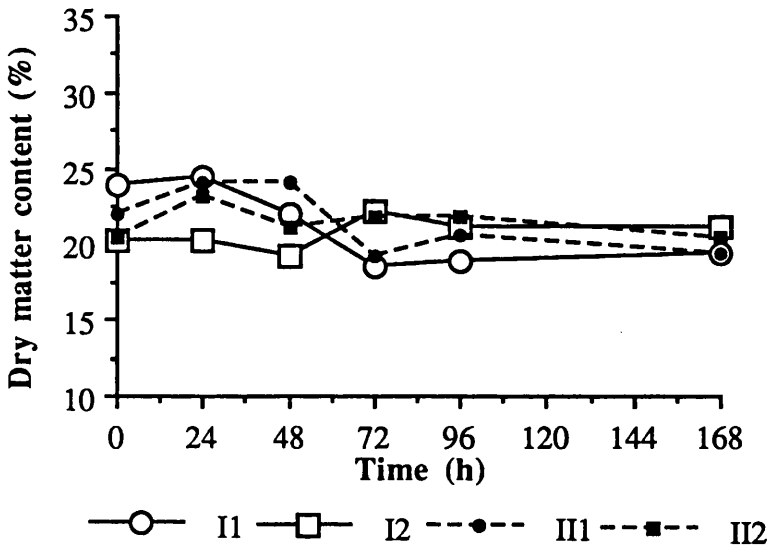

Figure 6-23. Faecal dry matter content following intravenous administration of oxytetracycline to ponies

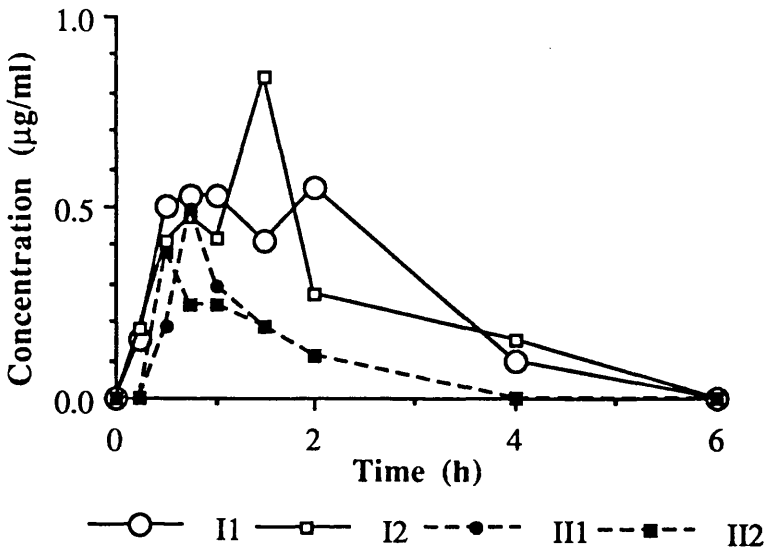

Figure 6-24. Plasma concentrations of oxytetracycline following oral administration to ponies

Parameter	I1	I2	II1	II2
t1/2 B2 (h)	0.37	0.48	0.21	0.24
t1/2 B1 (h)	1.07	1.20	0.63	0.89
C_{max} (µg/ml)	0.53	0.84	0.49	0.38
t_{max} (min)	45	90	45	30
AUC_{obs} (µg.h/ml)	1.59	1.48	0.51	0.48
$AUMC_{obs}$ (µg.h^2/ml)	2.85	2.81	0.64	0.60
AUC (µg.h/ml)	1.44	1.44	0.51	0.48
AUMC (µg.h^2/ml)	3.00	3.48	0.62	0.78
lag time (min)	-	-	9.24	6.00
MRT (h)	1.79	1.33	1.26	1.25
MAT (h)	-11.91	-12.37	-12.87	-12.88
F (%)	1.04	0.97	0.45	0.43

Table 6-6. Disposition kinetics of oxytetracycline in plasma following oral administration to ponies

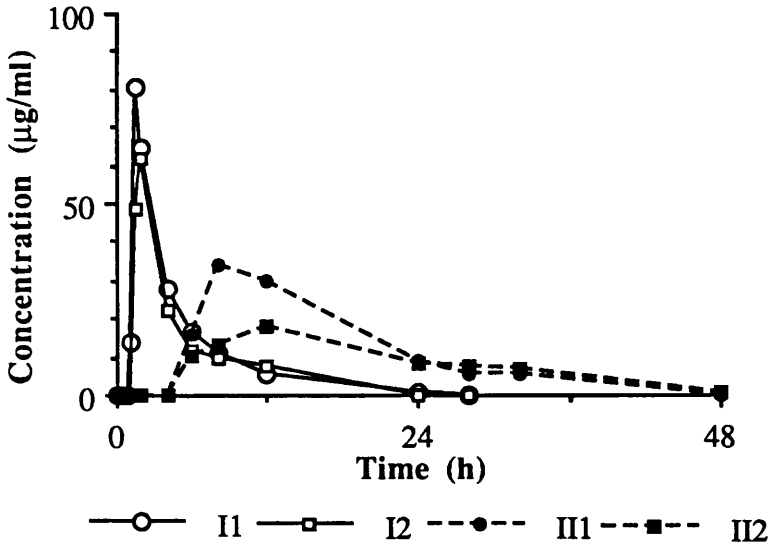

Figure 6-25. Caecal liquor concentrations of oxytetracycline following oral administration to ponies

Parameter	I1	I2	II1	II2
AUC_{obs} (µg.h/ml)	295.93	262.67	547.87	391.72
$AUMC_{obs}$ (µg.h^2/ml)	1546.10	1544.22	8704.68	7773.12
MRT (h)	5.22	5.88	15.89	19.84

Table 6-7. Disposition kinetics of oxytetracycline in caecal liquor following oral administration to ponies

maximum caecal liquor concentrations of 80.50, 61.74, 34.11 and 18.40 μg/ml were measured at 1.5, 2, 8 and 12 h after drug administration to ponies I1, I2, II1 and II2, respectively. The caecal liquor concentrations were much higher than the plasma concentrations of oxytetracycline following oral administration.

The disposition of oxytetracycline within the caecum was described using the observed AUC and AUMC to calculate the MRT (Table 6-7). The AUC, AUMC and MRT were much larger than those calculated for oxytetracycline in plasma following oral administration. There was a marked difference in MRT between the 2 animals, a fact that was also reflected in the difference in the time to maximum caecal liquor concentrations.

Faecal oxytetracycline concentrations following oral administration of oxytetracycline to ponies are shown in Figure 6-26, and the individual data is given in Appendix D (Table D56). Faecal drug concentrations were measured for up to 72 h in both animals on both occasions with the highest concentration in the 24 h samples.

6.5.3 Bacteriological examinations

Salmonella spp. were isolated and identified at 24 and 96 h after drug administration to pony I2. The organisms produced an alkaline slope, hydrogen sulphide and gas when inoculated into TSI slopes. The organisms agglutinated 'O', 'H' and 'B' antisera. The organisms were identified as *S. typhimurium* phage type 204c by the Salmonella Reference Laboratory. The isolates were susceptible to furazolidone and resistant to ampicillin, chloramphenicol, neomycin, oxytetracycline, potentiated sulphonamide, streptomycin, and sulfafurazole.

Clostridium difficile was selected for but not isolated.

Counts of viable bacteria in caecal liquor following oral administration of oxytetracycline are shown in Figures 6-27 to 6-31, and the individual data is given in Appendix D (Table D57).

There was an increase in the viable coliform count to 10^8-10^{11}/ml following oral administration of oxytetracycline to ponies (Figure 6-27). This was most marked (10^9-10^{11}/ml) at 24 h after drug administration to ponies I1, I2, I3, II1 and II2. In pony I1 the caecal liquor coliform count remained elevated (10^{10}/ml) at 48 h, fell at 72 h, and increased to 10^9/ml at 96 h. In pony I2 the coliform count was elevated to 10^8-10^9/ml at 168 and 336 h after drug administration. The alterations in the number of viable coliforms isolated from pony II3 at 24 h after drug administration were very slight (up to 10^7/ml).

There was an increase in the number of viable caecal streptococci (to 10^8-10^{11}/ml) isolated from all the ponies and this was most marked at 24 h after drug administration to ponies I1,

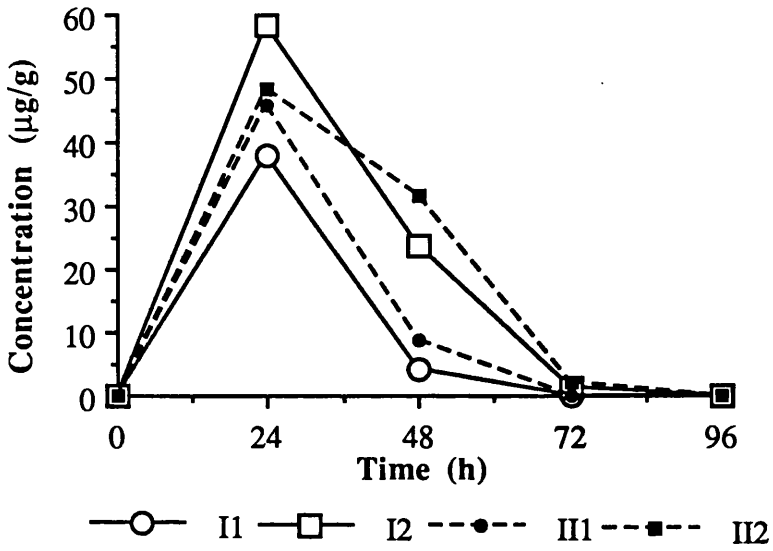

Figure 6-26. Faecal concentrations of oxytetracycline following oral administration to ponies

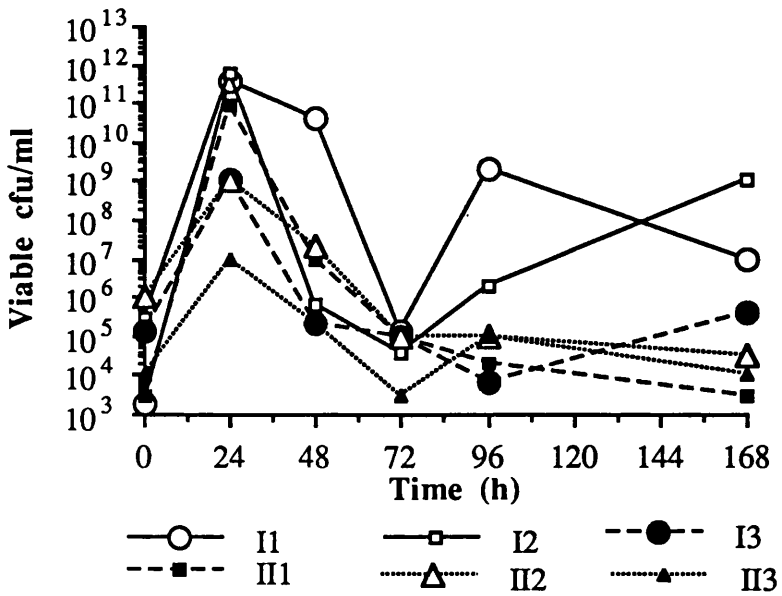

Figure 6-27. Counts of viable coliforms in caecal liquor following oral administration of oxytetracycline to ponies

I2 and II1 (Figure 6-28). The number of viable streptococci remained elevated (10^8-10^{11}/ml) in ponies I1, I2 and II1 at 48 h after drug administration and at 72 h (10^8/ml) in pony II1. All the counts were similar to the initial counts of 10^4-10^7/ml at 96 h after drug administration.

The number of viable lactobacilli in caecal liquor was increased to 10^9-10^{10}/ml at 24, 48 and 72 h in pony I1, at 48 h in pony I2, and at 24, 48 and 72 h in pony II1, compared with the initial values (Figure 6-29). In pony II2 the number of lactobacilli isolated was high (10^8-10^9/ml) prior to and at 48 h after drug administration. *Lactobacillus acidophilus and L. minutus* were isolated and identified using the API system.

There were no alterations in the number of viable *Bacteroides spp.* isolated from caecal liquor, except from pony II2 at 48 h after drug administration when there was a reduced count (10^8/ml) (Figure 6-30). *Bacteroides spp. (ovatus/thetaiotaomicron/uniformis), B. asaccharolyticus, B. bivius, B. fragilis/vulgatus, B. ovatus* and *B. thetaiotaomicron* were isolated and identified using the API system.

There were no *Clostridium spp.* isolated from caecal liquor prior to drug administration to ponies I1, I2 and II1 (Figure 6-31). The number of *Clostridium spp.* isolated from caecal liquor appeared to be quite high (10^6-10^7/ml) at 24 h in ponies I1, I2 and II1, and 48 and 72 h in ponies I1 and II1. The number of viable *Clostridium spp.* isolated from pony II2 remained low throughout the study. Bacteria identified using the API system were *Clostridium spp., C. clostridiiforme, C. butyricum* and *C. perfringens*. The latter was identified at 24 h after drug administration to ponies I1 (10^6/ml), I2 (10^7/ml), II1 (10^6/ml) and II2 (10^4/ml), and at 72 h after drug administration to pony II1 (10^4/ml).

Other bacteria identified using the API system were *Actinomyces israelii, Actinomyces naeslundii* and *Bifidobacter adolescentis.*

6.5.4 Caecal pH and SCFA concentrations and faecal SCFA concentrations

The caecal liquor pH measurements are shown in Figure 6-32, and the individual data is given in Appendix D (Table D58). In pony I1, caecal liquor pH was increased (>7.2) at 8, 12 and 48 h and reduced (<6.8) at 32, 36, 52, 56 and 96 h after drug administration. In pony I2, caecal liquor pH was low (<6.8) at 56 h after drug administration. There were no alterations in caecal liquor pH in pony I3. There was a reduction (<6.8) in caecal liquor pH at 48, 52, 56, 72 and 96 h in pony II1, at 56 and 96 h in pony II2, and on no occasions in pony II3.

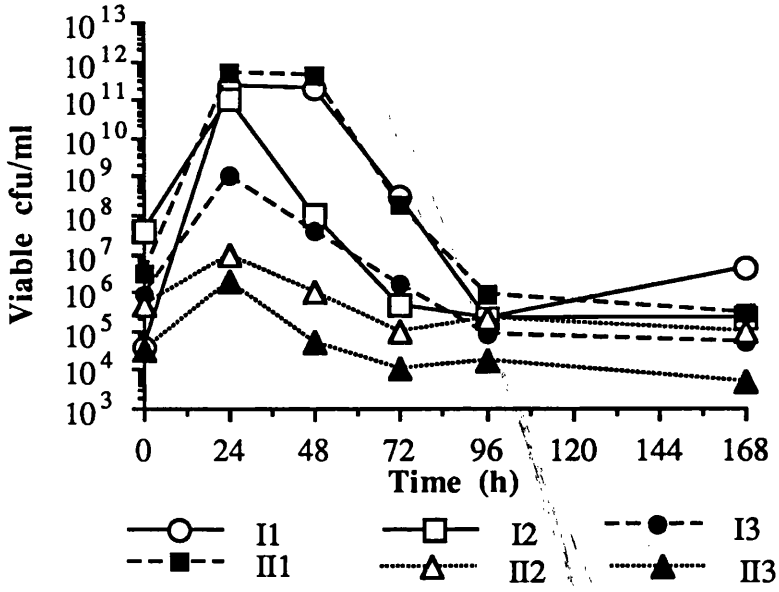

Figure 6-28. Counts of viable streptococci in caecal liquor following oral administration of oxytetracycline to ponies

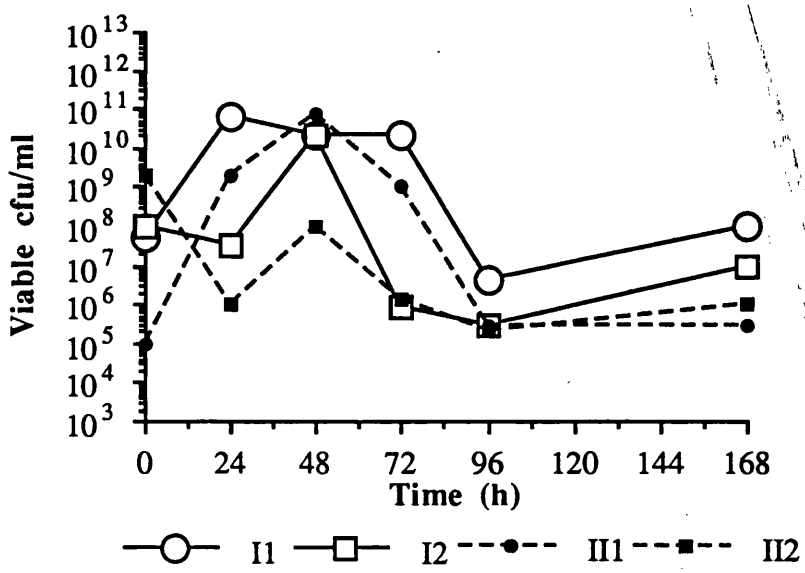

Figure 6-29. Counts of viable lactobacilli in caecal liquor following oral administration of oxytetracycline to ponies

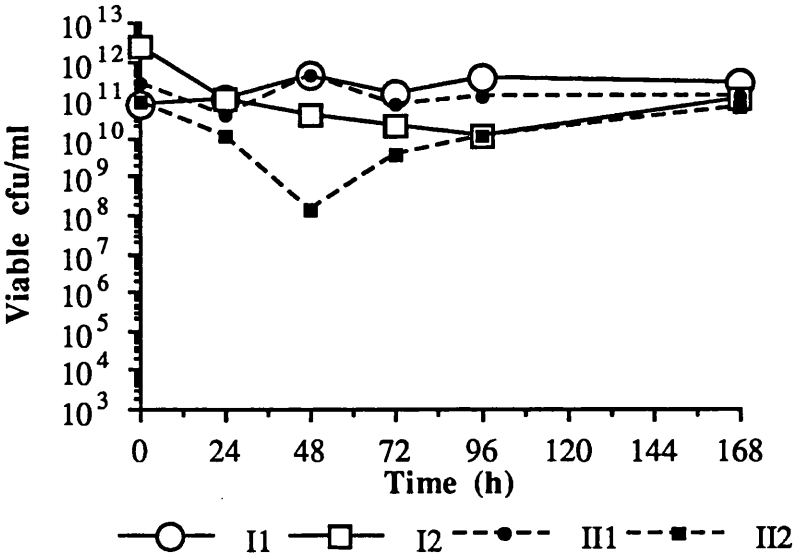

Figure 6-30. Counts of viable *Bacteroides spp.* in caecal liquor following oral administration of oxytetracycline to ponies

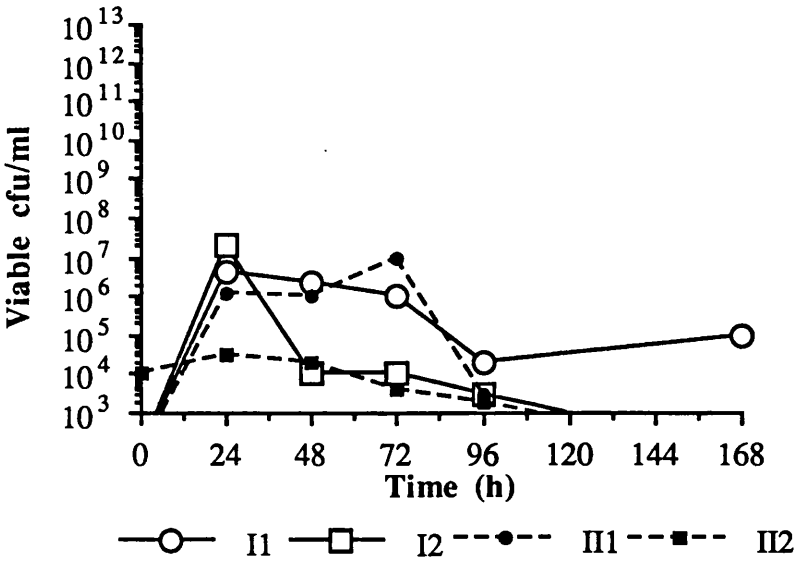

Figure 6-31. Counts of viable *Clostridium spp.* in caecal liquor following oral administration of oxytetracycline to ponies

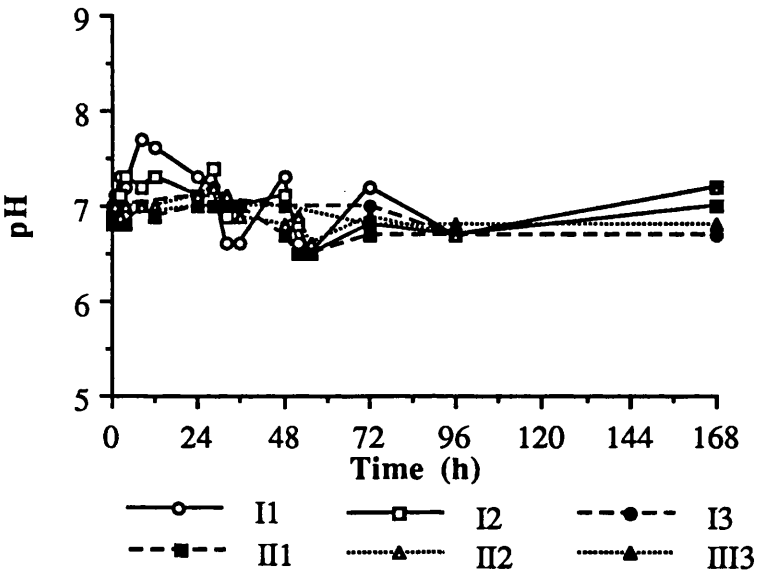

Figure 6-32. Caecal liquor pH following oral administration of oxytetracycline to ponies

Caecal liquor SCFA concentrations are shown in Figures 6-33 and 6-34, and the individual data is given in Appendix D (Tables D59a-D64a).

Caecal lactic acid concentrations (Figure 6-33) were increased to 5.5-53.9 mmol/l at 4, 6, 8, 12, 24, 28, 32 and 56 h, and were above the normal range of 0.0-24.4 mmol/l at 8, 12, 24 and 32 h after drug administration to pony I1. In pony I2, caecal lactic acid concentrations were elevated to 7.0-24.9 mmol/l at 4, 6, 8, 12, 24, 48, 52, 56 and 72 h, and were above the normal range at 8 h after drug administration. Lactic acid concentrations were slightly increased to 5.4 mmol/l at 168 h after drug administration to pony I3. In pony II1 caecal lactic acid concentrations were elevated to 5.6-46.4 mmol/l at 12, 24, 28, 32, 48, 52, 56, 72 and 96 h, and were above the normal range at 24 and 28 h after drug administration. Similarly, in pony II2 there was an increase in lactic acid concentrations to 7.0-32.0 mmol/l at 24, 28, 32, 48, 52, 56 and 72 h, and these were above the normal range at 56 h after drug administration. There were no marked increases in lactic acid concentrations in pony II3 following oral administration of oxytetracycline.

The total VFA concentrations fluctuated around the normal range of 24.4-109.2 mmol/l (Figure 6-34). There was a high total VFA concentration of 120.7 mmol/l prior to drug administration to pony I3. There were variations in the individual VFA concentrations that were outside the normal ranges following the oral administration of oxytetracycline. Acetic acid concentrations fell below the normal range of 12.6-64.5 mmol/l to 5.3-10.8 mmol/l at 28 h in pony I1, at 4, 6, 8 and 12 h in pony I2, and at 8 and 12 h in pony II1. There was an increase in the propionic acid concentrations to 32.3-37.7 mmol/l at 8 and 12 h in pony I1, and at 168 h in pony I3. There was a reduction in propionic acid concentrations to 2.3-4.1 mmol/l, compared with the normal range of 4.7-24.5 mmol/l, at 24 and 28 h in pony I1, at 24, 28, 32, 48, 52 h in pony I2, and at 28 and 32 h in pony II2. There was a reduction in the butyric acid concentrations to 3.4-4.5 mmol/l, compared with the normal range of 4.8-67.3 mmol/l, at 12 h in pony I2, and at 32 and 52 h in pony II2.

The proportions of acetic, propionic and butyric acids, expressed as a percentage of the total VFA concentrations, are given in Appendix D (Tables D59b-D64b). There were some variations in the ratios of acetic, propionic and butyric acids. There was a reduction in the percentage of acetic acid to 13.6-17.3%, compared with the normal range of 27.9-67.0%, at 12 h in pony I1, and at 4 and 8 h in pony I2. There was an increase in the percentage of propionic acid to 56.4-63.5%, compared with the normal range of 4.9-38.5%, at 12 h in pony I1, and at 4, 6, 8 and 12 h in pony I2. There was an increase in the percentage of propionic plus butyric acids to 76.0-86.4%, compared with the normal range of 27.1-72.1%, at 48 h in pony I1, at 4, 6, 8 and 12 h in pony I2, and at 72 h in pony I3.

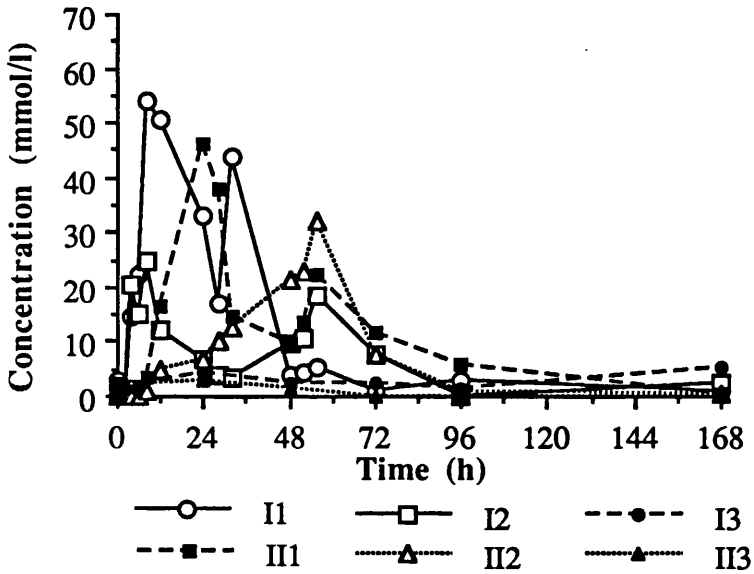

Figure 6-33. Lactic acid concentrations in caecal liquor following oral administration of oxytetracycline to ponies

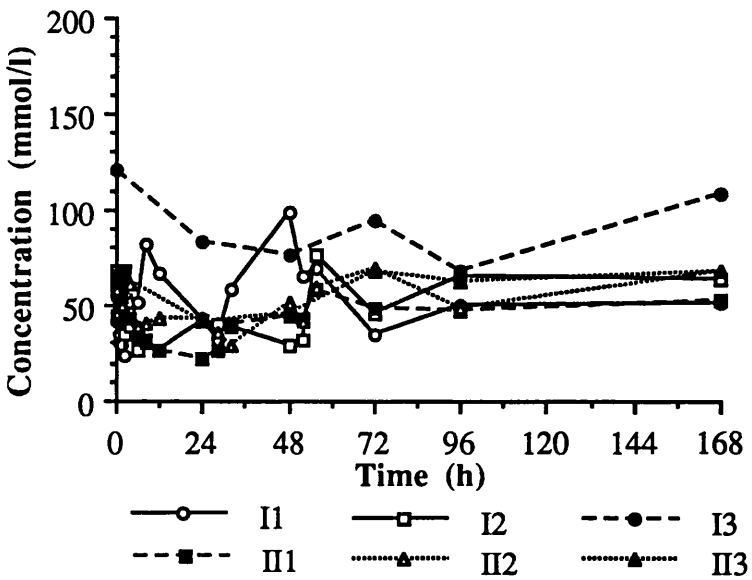

Figure 6-34. Total VFA concentrations in caecal liquor following oral administration of oxytetracycline to ponies

Faecal SCFA concentrations are shown in Figures 6-35 and 6-36, and the individual data is given in Appendix D (Tables D65-D70). There was an increase in lactic acid concentrations in faeces to 15.4-58.6 mmol/kg at 24 and 48 h in pony I1, at 48 and 72 h in pony I2, and at 24 h in pony I3, but only in pony I2 were the lactic acid concentrations outside the normal concentration range of 0.0-24.4 mmol/l (Figure 6-35). There was an increase in lactic acid concentrations to 5.0-65.4 mmol/kg at 24, 48, 72 and 168 h in pony II1 and at 24, 48, 72 and 96 h in pony II2, with the 24, 48 and 72 h concentrations on both occasions lying above the normal range. There were no marked alterations in lactic acid concentrations in pony II3. There were considerable variations in the total and individual VFA concentrations (Figure 6-36). In pony I1, the faecal total VFA concentration was lower than the normal range of 24.4-109.2 mmol/l prior to and at 168 h after drug administration. In pony I2, the total VFA concentration was lower than normal prior to drug administration due to a low acetic acid concentration at this time. In ponies I3, II1 and II3, the faecal total VFA concentrations were all within the normal range. In pony II1, the faecal total VFA concentrations were elevated above normal at 336 h. The VFA concentrations in faeces did not follow any particular trends following the oral administration of oxytetracycline to ponies.

6.5.5 Faecal dry matter content and consistency

The faecal dry matter content did not alter markedly following oral administration of oxytetracycline (Figure 6-37), and the individual data is given in Appendix D (Table D71). The faecal dry matter content was <17% prior to drug administration to pony I3 and at 24 h in pony II1. Faecal consistency was soft at 1.5 and 8 h in pony I1, and at 24, 48 and 72 h after drug administration to ponies I1, I2, II1 and II2.

6.5.6 Plasma biochemistry and haematological examinations

The plasma biochemistry results are given in Appendix D (Tables D72-D77). There was an increase in plasma urea concentrations up to 4.9-7.5 mmol/l following oral administration of oxytetracycline to pony I1. This was above the normal range at 24 h in pony I2. Fluctuations in other variables were around the normal ranges. The results of the haematological examinations are given in Appendix D (Tables D78-D83) and the parameters were all around the normal ranges.

6.6 Results of *in vitro* studies with oxytetracycline

6.6.1 Caecal liquor concentrations

A plot of concentrations (mean±SEM) of oxytetracycline in caecal liquor following incubation *in vitro* are shown in Figure 6-38, and the individual data is given in Appendix D

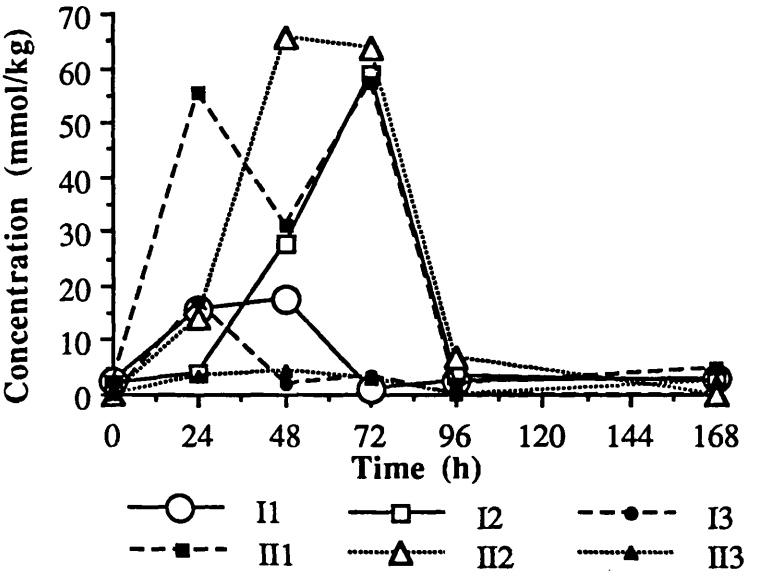

Figure 6-35. Lactic acid concentrations in faeces following oral administration of oxytetracycline to ponies

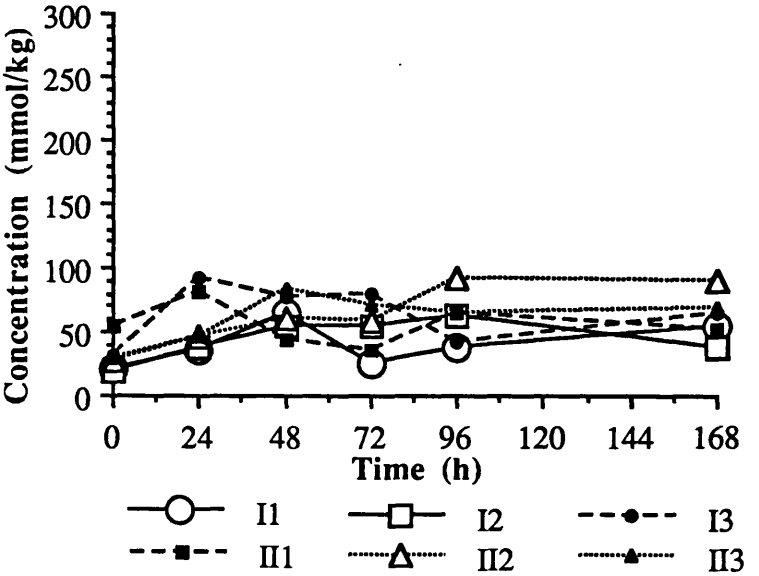

Figure 6-36. Total VFA concentrations in faeces following oral administration of oxytetracycline to ponies

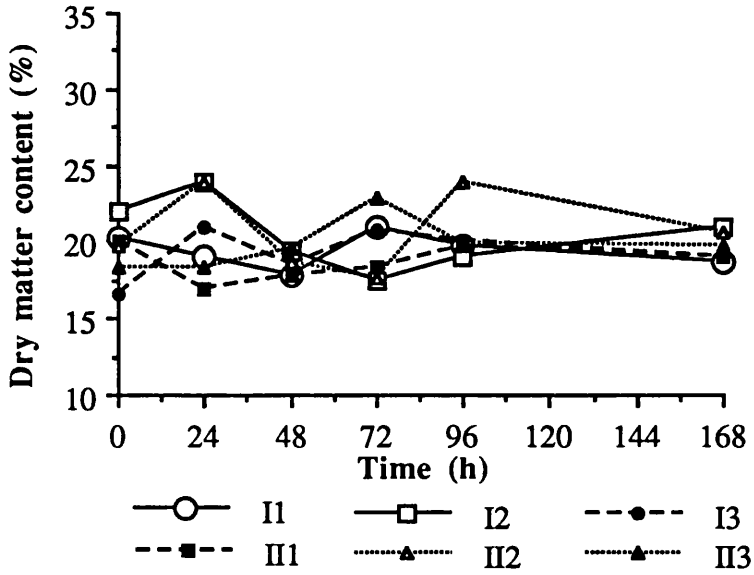

Figure 6-37. Faecal dry matter content following oral administration of oxytetracycline to ponies

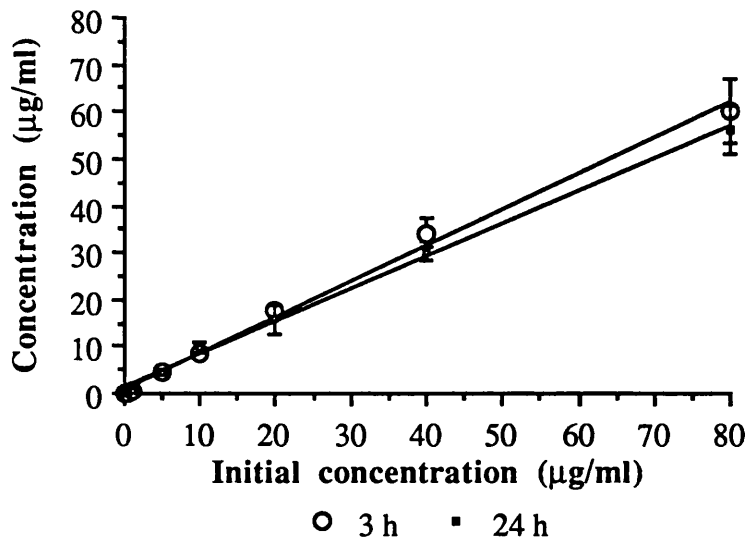

Figure 6-38. Concentrations (mean±SEM) of oxytetracycline in caecal liquor following incubation *in vitro* for 3 and 24 h

(Tables D84a and b). The limit of detection of the assays was poor; there were no inhibition zones at a concentration of 0.25 μg/ml. The largest reductions in concentration (zone diameter) were seen with the 1 μg/ml sample, probably due to limitations of the assay (zone diameter, limit of detection). In the other samples, following both 3 and 24 h incubation there was an average of *circa* 83 % of drug activity remaining.

6.6.2 Bacteriological examinations

The counts of viable bacteria (mean±SEM) in caecal liquor following *in vitro* incubation without or with oxytetracycline are shown in Figures 6-39 and 6-40, and the individual data is given in Appendix D (Table D85a and b). There were no alterations in the number of viable bacteria isolated following incubation of caecal liquor in the absence of oxytetracycline. There was an increase in the number of coliforms and *Clostridium spp.* isolated following 24 h incubation of caecal liquor with oxytetracycline at a concentration of 80 μg/ml.

6.6.3 SCFA concentrations

Lactic acid and total VFA concentrations (mean±SEM) in caecal liquor following *in vitro* incubation are shown in Figure 6-41, and the individual data is given in Appendix D (Tables D86-D90). There were no alterations in the mean lactic acid or mean total VFA concentrations with time or drug concentration.

6.6.4 Acid pH

Oxytetracycline concentrations (mean±SEM) remaining following *in vitro* incubation at pH 1.9 for 1 h are shown in Figure 6-42, and the individual data is given in Appendix D (Table D91). There were only very slight alterations in drug activity (mean concentration remaining *circa* 96 %) following incubation at pH 1.9.

6.7 Discussion

A variety of different dose rates have been used for oxytetracycline in the horse. Pilloud (1973) used a dose rate of 2.5 mg/kg bwt and, based on observed plasma concentrations and known MIC data, suggested that the dose rates recommended currently for horses by some authors and pharmaceutical companies, could well underestimate the amount of oxytetracycline necessary for successful treatment of some bacterial infections. Teske *et al.* (1973) and Baggot (1977b) administered oxytetracycline at a dose rate of 4.4 mg/kg bwt, and Brown *et al.* (1981) used a dose rate of 5 mg/kg bwt and were able to measure oxytetracycline for 48 h after drug administration using a microbiological assay. In a study

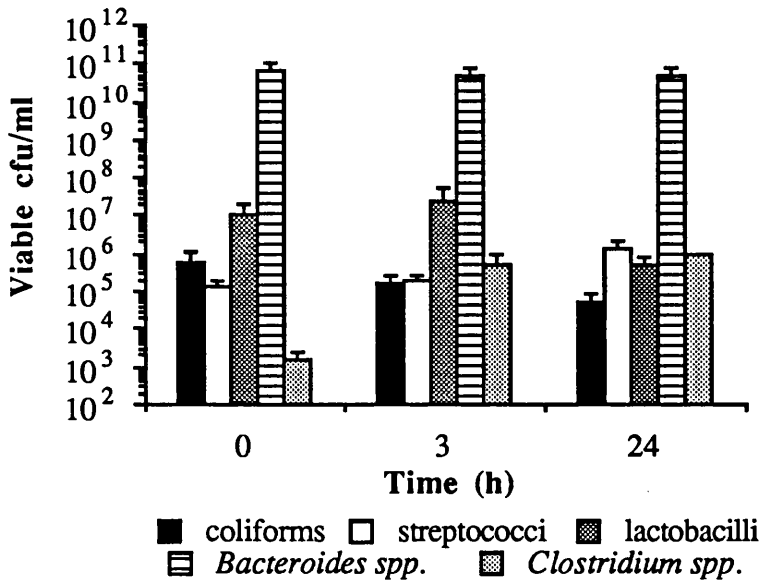

Figure 6-39. Counts of viable bacteria (mean±SEM) in caecal liquor following incubation *in vitro* for 3 and 24 h

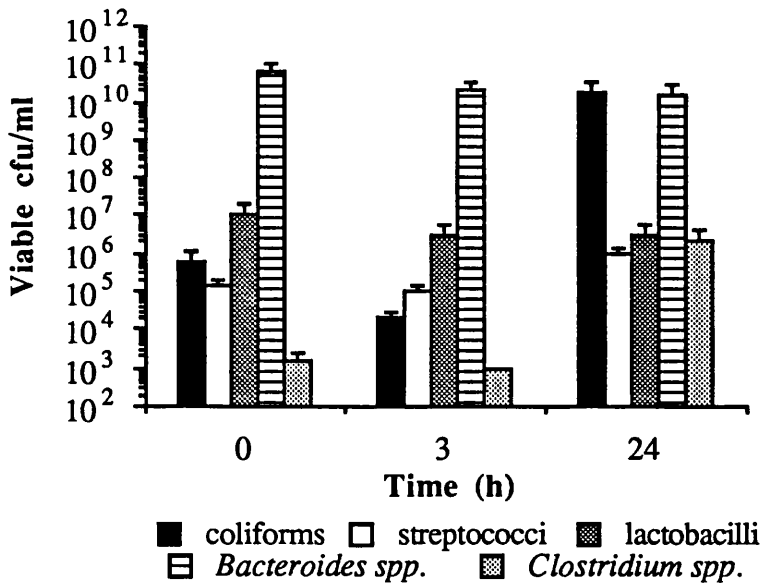

Figure 6-40. Counts of viable bacteria (mean ±SEM) in caecal liquor following incubation *in vitro* with 80 μg/ml oxytetracycline for 3 and 24 h

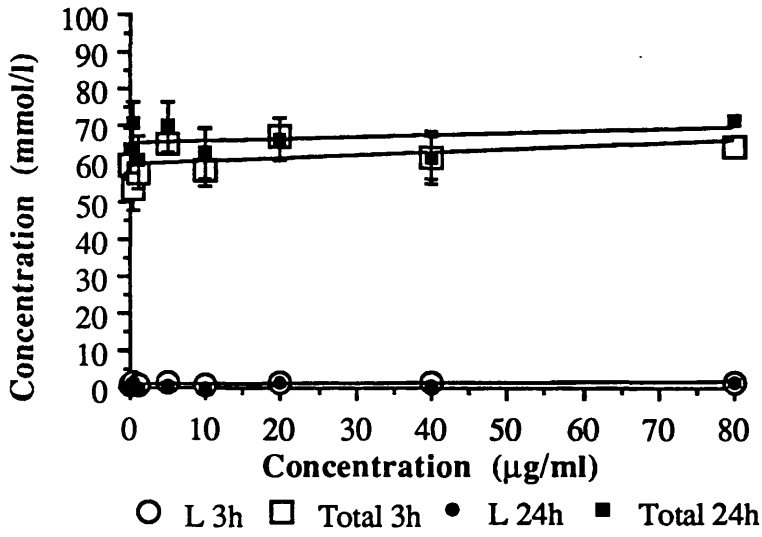

Figure 6-41. SCFA concentrations (mean±SEM) in caecal liquor following *in vitro* incubation with oxytetracycline for 3 and 24 h

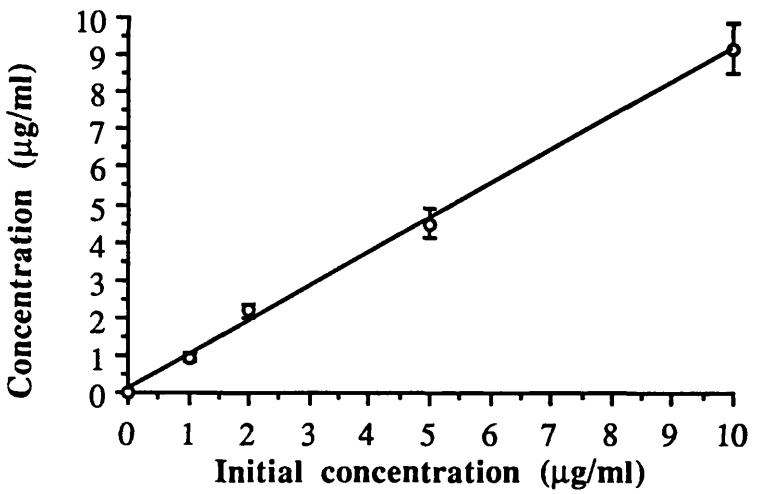

Figure 6-42. Concentrations (mean±SEM) of oxytetracycline following incubation *in vitro* at pH 1.9 for 1 h

to evaluate the tissue concentrations of oxytetracycline Larson and Stowe (1981) collected blood samples over a period of 4 h after intravenous administration of oxytetracycline at a dose rate of 10 mg/kg bwt and recorded plasma concentrations similar to the ones measured in the present study. The very long period over which oxytetracycline could be detected in the present study may reflect a more sensitive assay and a more appropriate sampling regimen than those used previously.

The dosage regimen necessary to maintain therapeutic plasma levels has been disputed. Prescott and Baggot (1988b) recommend a loading dose of 5 mg/kg bwt followed at 12 h intervals by maintenance doses at a rate of 3 mg/kg bwt to maintain therapeutic plasma concentrations of 1-5 μg/ml. The dosage regimen suggested by Van Miert (1988) utilizes the same interval between doses but suggested a higher dose rate of 5-10 mg/kg bwt. On the other hand, Bywater (1982b) suggested that serum concentrations of 0.5-1.0 μg/ml are appropriate, and indicated that a single daily intramuscular dose at a dose rate of 5-11 mg/kg bwt would be required to achieve this in cattle, dogs and horses. In the present study, the mean plasma concentrations measured were >1 μg/ml for 36 h in horses and ponies, and 24 h in donkeys, and were >0.5 μg/ml for 48 h in horses and ponies, and 24 h in donkeys. Thus, to maintain a therapeutic plasma concentration of 0.5-5.0 μg/ml the dosage interval of oxytetracycline hydrochloride, when administered intravenously at a dose rate of 10 mg/kg bwt, should be 48 h in horses and ponies, and 24 h in donkeys. This does not take into account the putative post-antibiotic effect.

It is interesting that the harmonic mean of elimination half-life was much higher in horses and ponies, than it was in donkeys. The variation in elimination half-life may be due to an inter-species variation in drug elimination of oxytetracycline since horses and ponies are *E. caballus* whilst donkeys are *E. asinus*. Kirkwood and Widdowson (1990) demonstrated that there was a linear relationship between the elimination half-life of oxytetracycline and the bodyweights of different species. The bodyweights of the animals used in the present study were applied to the equation described by Kirkwood and Widdowson (1990), however the estimates of elimination half-life were lower than calculated in the present study for horses and ponies (9.20-9.63 h and 7.37-8.78 h, respectively) and higher than calculated for donkeys (7.37-7.79 h). Thus, there appeared to be a greater difference in elimination half-life of oxytetracycline in horses, ponies and donkeys than could be explained merely by inter-species variation in bodyweight.

There were marked inter-species differences in other pharmacokinetic parameters. The mean AUC and AUMC were higher in horses than they were in donkeys. In addition, the mean CL_b was much slower in horses than it was in donkeys. This did not seem to be due to a difference in clearance of drug by the liver since the elimination half-life of bromsulphalein

was similar in horses and donkeys. A similar difference was demonstrated by Kinabo and Bogan (1989) following the oral administration of the flukicidal drug triclabendazole to horses, ponies and donkeys. A similar dosage regime of oxytetracycline could be used in horses and ponies, but donkeys, due to differences in pharmacokinetics, would require more frequent dosing to maintain therapeutic plasma concentrations of oxytetracycline.

The penetration of oxytetracycline into the large intestine following intravenous administration was estimated from the observed AUCs in caecal liquor and plasma, the estimated volume of caecal contents, and the estimated plasma volume (Swenson, 1977), and was expressed as a percentage. This was quite variable ranging from 4.32-31.43%, but was less than 17% in 6 of the 7 ponies. Thus it appears that up to 31.43% of oxytetracycline may be in the gastrointestinal lumen following intravenous administration. Although it is likely that a figure of between 4 and 17% of the total dose of oxytetracycline is eliminated into the gastrointestinal lumen following a single intravenous administration. It would be interesting to examine biliary excretion of oxytetracycline in the equine either by comparing gastrointestinal elimination with renal drug elimination or by using direct techniques, *i. e.* by measurement of biliary excretion rates.

The systemic availability of oxytetracycline following oral administration to ponies was very low. This was not due to the destruction of the drug either by acid pH or by the gastrointestinal contents. The poor systemic availability of oxytetracycline in ponies was quite surprising since in other species, such as the dog and cat, most tetracyclines are absorbed adequately from the gastrointestinal tract. Although, oxytetracycline was absorbed from the gastrointestinal tract following oral administration, and the plasma concentrations were in some cases sufficient to treat infections caused by susceptible bacteria, the fact that the MRT was short means that frequent dosing would be required to maintain adequate therapeutic concentrations in plasma.

The faecal samples from horses, ponies and donkeys, following intravenous administration of oxytetracycline, had similar concentrations of oxytetracycline which could be measured for 96 h in all three groups. This was an unexpected result since plasma concentrations of oxytetracycline were measurable for 96 h in horses, 72 h in ponies and 48 h in donkeys. Moreover, the faecal concentrations were greater than the simultaneous plasma concentrations in all the samples from the horses, ponies and donkeys. Similarly, there was a large amount of oxytetracycline measured in the caecal liquor (AUC_{obs} 262.67-547.87 μg.h/ml) following intravenous administration to ponies with cannulated caecal fistulas. This was not especially surprising since biliary concentrations can average at least five to ten times higher than simultaneous plasma concentrations of oxytetracycline (Owen, 1975).

In the present study, *S. typhimurium* phage type 204c was isolated from pony I2 on two separate occasions following oral administration of oxytetracycline. Owen *et al.* (1983) stated that the use of oxytetracycline prolonged the stress-activated excretion of *Salmonella spp.*, and advised that oxytetracycline should not be administered to ponies after stress (transport), to ponies with diarrhoea, or to ponies which are known carriers of *Salmonella spp.* infection. However, the *Salmonella spp.* was isolated from only 1 animal in the present study, and the administration of oxytetracycline was repeated on a further occasion and failed to activate further excretion of the organism, despite meticulous attempts to isolate it. Other authors have reported a series of cases of *S. typhimurium* infection where the organism was excreted for less than 1 month and where there was no subsequent excretion of the organism for at least 1 year (Smith *et al.*, 1980). In *Equidae*, *Salmonella spp.* infection is sporadic, and the clinical signs can range from asymptomatic infection, or fever, anorexia and depression, to severe acute diarrhoea or septicaemia (Smith, 1981). In the present case, there was anorexia and slight diarrhoea which may have been attributed to *S. typhimurium* infection but may have been due to drug administration since the clinical signs were seen in the absence of the isolation of *Salmonella spp.*, too. Neither prolonged excretion of *Salmonella spp.* nor neutropaenia were recorded in the present study. Hird *et al.* (1984) and Hird *et al.* (1986) noted that there was a predisposition to salmonellosis in horses in which the treatment had involved a variety of factors, such as nasogastric intubation (3.9 or 2.85 times greater risk) and oral antimicrobial therapy (1.28 times greater risk). In the present study, *S. typhimurium* was a hospital acquired infection. A number of equine cases had been admitted to the Glasgow University Veterinary School with *Salmonella spp.* infection of the same serotype and phage type identified here. As an adjunct to the present study, the selective isolation of *Salmonella spp.* was carried out from the terminal ileum, caecum and mesenteric lymph nodes of a series of 12 ponies, at slaughter, which were kept in the same area as the ponies I and II. However, no *Salmonella spp.* were isolated. Perhaps, the type of treatment involved in the present study predisposed the animals involved to the development of *Salmonella spp.* infection. Although, it is strange that the organism was isolated from one animal only and that there were no obvious clinical signs, apart from depression and anorexia, and that the organism was not excreted for a prolonged period.

A variety of different serotypes of *Salmonella spp.*, including *typhimurium*, *krefeld*, *saint-paul*, *typhisuis* and *dublin*, have been isolated from animals (Ikeda *et al.*, 1986) with group B predominating (Benson *et al.*, 1985). A variety of different *Salmonella spp.* have been isolated from the equine gastrointestinal tract with the prevalence ranging from 0.36-27 % (Al-Mashat and Taylor, 1986, Gibbons, 1980, Morse *et al.*, 1976). Over 40 different serotypes of *Salmonella spp.* have been isolated from *Equidae* and around 66.31% of these

isolates were *S. typhimurium* whilst other serotypes, such as *S. enteritidis*, made up less than 10% of the isolates (Morse *et al.*, 1976). Therefore, it is not surprising that *S. typhimurium* was the serotype isolated in the present study, particularly in view of the preceding antimicrobial therapy.

In one survey, greater than 60% of *Salmonella spp.* isolates were resistant to ampicillin, kanamycin, neomycin, streptomycin and the sulfonamides (Ikeda *et al.*, 1986). However, resistance to chloramphenicol, gentamicin and the potentiated sulphonamides was infrequent except for serotypes *krefeld* and *saint-paul*. A second study reported that most *Salmonella spp.* isolated from large animals were resistant to ampicillin and tetracycline (Benson *et al.*, 1985). Thus, treatment of *Salmonella spp.* infection must follow careful antimicrobial susceptibility testing of isolates, although a knowledge of the common resistance patterns is useful. In the present study, *S. typhimurium* was resistant *in vitro* to all the standard antimicrobial agents tested against enteric pathogens, except furazolidone, and to erythromycin, benzylpenicillin, oxytetracycline and lincomycin (P. Sarasola, unpublished data). In addition, MIC data from the isolate of *S. typhimurium* in the present study reflected resistance *in vitro* to ampicillin (>128 μg/ml), mezlocillin (>128 μg/ml), amoxycillin/clavulanic acid (32/16 μg/ml), cephalothin (16 μg/ml), cefoxitin (8 μg/ml), tetracycline (>16 μg/ml), carbenicillin (>128 μg/ml), ceftizoxime (<16 μg/ml), and trimethoprim/sulphonamide (>16/>304 μg/ml) and susceptibility to amikacin (<1 μg/ml), ciprofloxacin (<0.06 μg/ml), gentamicin (<0.5 μg/ml), and norfloxacin (<1 μg/ml) (P. Sarasola, unpublished data). The resistance pattern of the isolate in the present study was similar to other *S. typhimurium* isolates of equine origin.

The coliform counts recorded in the present study fall into both the range of 10^3-10^4/g faeces described by Smith (1965), and 10^6/g faeces described by White and Prior (1982), but were much lower than the control values used by Garner *et al.* (1978). Andersson *et al.* (1971) recorded an increase in the number of coliforms isolated from faeces 1-2 days after intravenous administration of oxytetracycline to horses at a dose rate of 15-26 mg.kg bwt. White and Prior (1982) recorded viable coliform counts of around 10^9/g faeces following repeated oral administration of oxytetracycline at a dose rate of 40 mg/kg bwt. Similarly, in the present study there was an increase in the number of coliforms isolated from donkey faeces at 72 and 96 h after intravenous administration of oxytetracycline. There was an increase in caecal coliform counts up to 10^9/ml at 24 h and for up to 168 h after oral administration of oxytetracycline to ponies with cannulated caecal fistulas. In addition, there was an increase in the number of coliforms isolated from caecal liquor following incubation *in vitro* in the presence of a high concentration of oxytetracycline.

Andersson *et al.* (1971) and White and Prior (1982) found that there were significant alterations in the number of streptococci isolated from faeces following administration of oxytetracycline. There were no marked increases in the number of streptococci isolated following intravenous administration, but there were increases in the number of viable streptococci isolated following oral administration of oxytetracycline in the present study. However, these changes were not reflected in the *in vitro* study.

There was an apparent increase in the number of lactobacilli isolated following intravenous administration of oxytetracycline to horses, and following oral administration of oxytetracycline to ponies contrary to the findings of White and Prior (1982). There were no alterations in the number of lactobacilli isolated in the *in vitro* study.

White and Prior (1982) examined the faecal flora of horses after the oral administration of oxytetracycline, and compared this to the effect of the oral administration of a trimethoprim/sulphadiazine combination. They isolated *C. perfringens* on 4 out of 8 occasions only, and described this as an alteration in the faecal flora since the isolations occurred after drug administration. *Clostridium spp.* were counted in the present study. There was an increase in number of *Clostridium spp.* isolated at 96 h after intravenous administration of oxytetracycline to horses, but *C. perfringens* was not identified. However, there was an increase in the number of *Clostridium spp.* isolated from caecal liquor for up to 72 h after oral administration of oxytetracycline to ponies with cannulated caecal fistulas. *Clostridium perfringens* was identified at 24 h after drug administration to ponies with cannulated caecal fistulas. *Clostridium spp.* were not isolated on all occasions partly due to the limit of detection of the assay, a fact that was also noted in the study by White and Prior (1982). The *Clostridium spp.* identified in the present study (*C. perfringens*, *C. clostridiiforme* and *C. butyricum*) have been isolated from enteric lesions of horses (Al-Mashat and Taylor, 1986).

Andersson *et al.* (1971) reported an increase in the faecal pH from 6.1-6.5 to 6.9-8.1 following intravenous administration of a massive dose of oxytetracycline to horses. The control caecal pH range noted by Garner *et al.* (1978) was around 7.2. In the present study there was a modest reduction in the caecal liquor pH following oral administration of oxytetracycline to ponies. This result may not be all that surprising since there were alterations in the SCFA concentrations in caecal liquor.

The marked alterations in SCFA concentrations were in lactic acid concentrations, and not in VFA concentrations, in which there were no obvious trends. There was an increase in the mean lactic acid concentration in faeces at 48 h in horses and 72 h in donkeys following intravenous administration of oxytetracycline. There were increases in both caecal and faecal

lactic acid concentrations following both intravenous and oral administration of oxytetracycline to ponies with cannulated caecal fistulas. The increase in lactic acid concentrations could be explained by the increase in the number of lactic acid producing bacteria, such as streptococci, lactobacilli and *Clostridium spp.* that were isolated. Both D- and L-lactic acid are produced by gastrointestinal bacteria although only the latter is utilized in mammals. It would have been useful to separate D- and L-lactic acid. The increased lactic acid concentrations in the present study occurred at variable times following intravenous or oral administration of oxytetracycline, although they always occurred following the peak drug concentration. Systemic acidosis, important in a number of equine diseases, may be due to the absorption of lactic acid from the gastrointestinal tract or to a reduction in plasma bicarbonate ion concentrations due to the movement of these ions from the plasma into the gastrointestinal lumen to act as a buffer. Lactic acid that is not absorbed from the gastrointestinal lumen may play a role in the development of osmotic diarrhoea, either by an increase in the secretion or a reduction in the absorption of water and electrolytes.

Prescott and Baggot (1988b) reported that a mild pastiness of the faeces is often recorded after administration of oxytetracycline to horses. Cook (1973) postulated that the administration of the tetracyclines is associated with the development of diarrhoea in horses but there are often other factors, such as anaesthesia or surgery, involved. In the present study, there were slight alterations in the faecal dry matter content particularly following intravenous administration of oxytetracycline to horses, ponies and donkeys. Following oral administration of oxytetracycline, faecal consistency was softer than normal, however there were no marked alterations in the faecal dry matter content.

Andersson *et al.* (1971) described a clinical syndrome, with signs of diarrhoea, apathy and anorexia, which occurred 3-4 days after an intravenous administration of a massive dose of oxytetracycline to horses. Similar clinical signs were described by White and Prior (1982) following repeated oral administrations of a high dose (40 mg/kg bwt) of oxytetracycline, and in the present study, following oral administration of a single dose of oxytetracycline at a dose rate of 10 mg/kg bwt. It may be that repeated administrations or administration at a higher dose rate would precipitate the clinical syndrome described previously.

There was an increase in the plasma urea concentrations following oral administration of oxytetracycline to ponies in the present study. This alteration in clinical chemistry was noted by Andersson *et al.* (1971) although none of the other changes noted by these authors were observed in the present study.

In man, the tetracyclines have been recorded as inducing diarrhoea by altering the normal gastrointestinal microflora and allowing superinfections, such as *Salmonella spp.* infection, to take place (Finger and Wood, 1955, Weinstein *et al.*, 1954). In the present study, there

were alterations in the normal gastrointestinal microflora, particularly following oral administration of oxytetracycline. The presence of *Salmonella spp.* infection was identified in one animal. In addition, there were alterations in the concentrations of microbial fermentation products, particularly lactic acid concentrations, in the gastrointestinal lumen.

A single intravenous administration of oxytetracycline hydrochloride at an appropriate dose rate, such as 10 mg/kg bwt, did not appear to pose a severe risk to gastrointestinal microbial function. A study of repeated administrations would provide further information on the potential and reported gastrointestinal side effects. However, oxytetracycline should be used with caution in the equine since there were increases in caecal liquor and faecal lactic acid concentrations following a single intravenous administration. In view of the clinical signs, low plasma concentrations, and the alterations in the commensal bacterial population in the gastrointestinal tract following a single oral administration of oxytetracycline hydrochloride, this route of administration should probably be avoided.

7 General Discussion

The present study set out to evaluate the plasma disposition and pharmacokinetics of penicillin G, ampicillin, amikacin and oxytetracycline following intravenous administration to horses, ponies and donkeys. Penicillin G and ampicillin are eliminated primarily by the kidneys. In the present study, up to 1.95% of the penicillin G and up to 3.66% of the ampicillin appeared to be eliminated in the bile or passed into the lumen through the gastrointestinal mucosa from the systemic circulation. The aminoglycosides are eliminated by the kidneys and, in the present study, no amikacin was detected in the gastrointestinal contents following intravenous administration. In comparison, the tetracyclines are eliminated by the kidneys and in the bile, and in the present study there was a large amount of oxytetracycline (up to 16.95%) eliminated into the gastrointestinal tract following intravenous administration.

There may be inter-species differences in drug disposition and pharmacokinetics which affect the calculation of optimum dose rates and regimes. This may be due to differences in the total body water, extracellular fluid volume, fat content, or metabolism. The donkey (*Equus asinus*) is used widely throughout the world as a draught animal. It has been suggested that there are differences between the disposition and pharmacokinetics of drugs in the donkey and in the horse and pony (*Equus caballus*) which manifest as a lower AUC and a higher CL_b (Kinabo and Bogan, 1989). This was corroborated in the present study where the CL_b of ampicillin, and possibly oxytetracycline, was faster in donkeys than in horses and ponies. Differences in drug elimination may result in differences in the optimal dosage requirements. However, the implications of the results of the present study are limited by the small number of animals used. It would be interesting to compare the total body water, and the renal and hepatic clearance rates in horses and donkeys. Bromsulphalein clearance involves active uptake into hepatocytes, conjugation to glutathione and biliary excretion. In the present study, bromsulphalein was used as an indicator of hepatic clearance and the elimination half-life of bromsulphalein was within the range (2.0-3.7 min) quoted normally for the horse (Anon, 1986). This suggests that the biliary clearance rate was similar in horses and donkeys. It would have been useful to measure the urinary excretion of the antimicrobial agents since a large proportion of the antimicrobial agents studied were eliminated by the kidneys. In a recent study, the aminoglycoside gentamicin was used as an indicator of renal excretion in the horse since the aminoglycosides are eliminated exclusively by renal filtration (Clarke *et al.*, 1992). However, in the present study the CL_bs of the aminoglycoside amikacin were similar in horses and donkeys but approximately twice as fast in ponies.

The differences in the pharmacokinetics of penicillin G, ampicillin, amikacin and oxytetracycline reflect differences in the physicochemical nature of the four antimicrobial agents. The penicillins are water soluble and polar and are eliminated rapidly by the kidneys, and have an apparent volume of distribution similar to the extracellular fluid volume of the animal (23% bwt). In the present study, penicillin G had a short elimination half-life in horses, ponies and donkeys (38.95, 27.25 and 31.52 min, respectively), a short distribution half-life (5.85, 2.52 and 4.28 min, respectively), a Vd_{SS} similar to the extracellular fluid volume of the animal (362.32, 314.05 and 204.53 ml/kg, respectively) and a high CL_b (514.46, 505.36 and 462.86 ml/h.kg, respectively). Similarly, the semisynthetic penicillin, ampicillin had a relatively short elimination half-life in horses, ponies and donkeys (60.88, 52.34 and 42.80 min, respectively), a short distribution half-life (14.79, 6.62 and 5.86 min, respectively), a Vd_{SS} similar to penicillin G (345.31, 260.44 and 422.49 ml/kg, respectively) and a high CL_b (229.67, 257.68 and 418.80 ml/h.kg, respectively). The aminoglycosides are poorly lipid soluble and highly polar compounds and therefore distribute in a volume similar to the extracellular fluid volume although they have been shown to attain measurable concentrations in intracellular compartments (Bowman *et al.*, 1986, Orsini *et al.*, 1985). Amikacin had a slightly longer elimination half-life than the penicillins in horses, ponies and donkeys (2.84, 1.60 and 1.93 h, respectively), a short distribution half-life (6, 12 and 3 min), a Vd_{SS} similar to but slightly smaller than the penicillins (206.58, 162.73 and 150.21 ml/kg, respectively) and a slower CL_b (45.19, 82.37 and 57.98 ml/h.kg, respectively). Oxytetracycline distributes widely in the body and reaches high concentrations in bronchial fluid, synovial fluid, peritoneal fluid, lung and renal tissue (Brown *et al.*, 1981, Larson and Stowe, 1981). In the present study, oxytetracycline had a long elimination half-life in horses, ponies and donkeys (11.69, 11.77 and 5.40 h, respectively), a reasonably short distribution half-life (9, 13.8 and 17.4 min, respectively), an apparent Vd_{SS} greater than the extracellular fluid volume of the animal (615.97, 956.26 and 649.27 ml/kg, respectively) and a slower CL_b than the penicillins (39.31, 60.77 and 91.37 ml/h.kg, respectively). The CL_bs of amikacin and oxytetracycline were slower than for penicillin G and ampicillin. This may be due to differences in the elimination of the antimicrobial agents by the kidney or to differences in the distribution of the agents within the body. It is interesting that both amikacin and oxytetracycline, which have been shown to achieve therapeutic concentrations in body fluids such as peritoneal and synovial fluid, had slower CL_bs than the penicillins. Amikacin is polar and has a low lipid solubility, whilst oxytetracycline is much more lipid soluble and hence has a larger Vd_{SS} reflecting apparently greater distribution within the body. The greater distribution of oxytetracycline or enterohepatic circulation, *i. e.* reabsorption of drug from the intestinal lumen following biliary elimination, may have resulted in the longer elimination half-life.

In the present study, an evaluation of the systemic availability of penicillin G, ampicillin, amikacin and oxytetracycline was made, following administration by nasogastric tube to ponies, to assess their potential application in systemic antimicrobial therapy in the equine. Following oral administration, a drug must pass from the gastrointestinal lumen through the gastrointestinal wall and the liver prior to entering the systemic circulation. An agent may be absorbed poorly due to insufficient time for absorption, decomposition within the gastrointestinal tract, poor availability for absorption (*e. g.* bound to food), or biliary elimination. An agent may be eliminated by the liver prior to entering the systemic circulation, the so-called first pass effect. The absorption time may be insufficient for polar compounds, such as aminoglycosides which are essentially unabsorbed. In addition, only unionized agents are absorbed normally, and most drugs are ionized in the gastrointestinal tract. The penicillins are acidic and are more likely to be absorbed from an acidic environment such as the stomach or the upper small intestine. The aminoglycosides have a high molecular weight and are polar and poorly lipid soluble resulting in a very low rate of absorption. The tetracyclines are amphoteric but tend to be basic and are more likely to be absorbed at the higher pH that occurs in the distal small intestine. In the present study, penicillin G, ampicillin and oxytetracycline were absorbed rapidly and attained peak plasma concentrations at 0.5, 0.5-0.75 and 0.5-1.5 h, respectively. The peak plasma concentrations may have been measured slightly later for oxytetracycline because it was absorbed at a different region of the small intestine. The systemic availability of these antimicrobial agents was poor (0.12-0.34, 1.20-5.54 and 0.43-1.04%, respectively). In the present study, the apparent half-life of the terminal phase (t1/2 B1) for penicillin G, ampicillin and oxytetracycline was shorter than the elimination half-life calculated following intravenous administration. The elimination half-life cannot be calculated directly from the terminal phase of the plasma concentration versus time data following oral drug administration because it is a composite of the absorption, distribution and elimination of a drug. In some instances, where the systemic availability is limited by the rate of absorption, the apparent half-life of the terminal phase is in fact the absorption half-life, the so-called flip-flop phenomenon. It is not particularly surprising that the systemic availability of penicillin G appeared to be lower than the systemic availability of ampicillin since penicillin G is hydrolyzed (around 85%) at acid pH. In man and young animals, it is recommended that milk and antacids are avoided during oral administration of the tetracyclines because the latter complex with polyvalent cations such as calcium and magnesium ions. The systemic availability of oxytetracycline was low following oral administration and this may have been due to binding of the drug to feed. Although, the calculated values in the present study may be erroneous due to differences between the preparations used for intravenous and oral administration in the present study. It would have been useful to have examined the binding of oxytetracycline to hay *in vitro*. In the present study, 92.9% of the penicillin G and 59.2% of the ampicillin

were bound to hay following incubation *in vitro* at pH 7.0. Around 19.8, 5.0 and 17.0% of the activity of penicillin G, ampicillin and oxytetracycline, respectively, was lost following *in vitro* incubation in caecal liquor for 3 h. It appears that the low systemic availability of antimicrobial agents following oral administration to equines may be partly due to acid hydrolysis, binding to feedstuffs and destruction within the gastrointestinal lumen. It would be interesting to re-examine the systemic availability of these three antimicrobial agents following oral administration to animals that had feed withheld.

The interactions of antimicrobial agents with the gastrointestinal microflora, and the effects of the route of administration on the magnitude of the gastrointestinal side effects were evaluated in the present study. Much of the work relied on the use of two ponies with cannulated caecal fistulas. Intestinal cannulation/fistulation is a technique that has been used in many of the studies of digestion in animals. Consequently, a number of different techniques have been described (Alexander, 1970, Boyd, 1985, Lowe *et al.*, 1970, Roberts and Hill, 1974, Simmons and Ford, 1988). It is arguable whether the gastrointestinal microclimate of a fistulated animal is normal. Some authors have reported alterations in digestion following caecal fistulation whilst others reported no differences in the rate of passage of digesta or digestibility of feed (Lowe *et al.*, 1970, Pulse *et al.*, 1973). Mackie and Wilkins (1988) commented that the total viable counts of bacteria were higher in fresh *post mortem* samples compared with samples taken from animals with caecal fistulas. In their study, a total of 2.59 x 10^9/g were isolated from the caecal contents of horses and this was not dissimilar to the results of the present study (>10^9 *Bacteroides spp.*/ml). Moreover, there were higher numbers of viable bacteria (*Bacteroides spp.*) isolated on anaerobically incubated horse blood agar in the present study than Mackie and Wilkins (1988) isolated on anaerobe blood agar containing defibrinated horse blood (5.25 x 10^8/g of contents). It appears that the gastrointestinal microenvironment of animals with caecal fistulas provides an adequate representation of the caecal environment in normal ponies.

Clostridium perfringens is implicated in the pathogenesis of so-called colitis 'X' in the equine (Swerczek, 1979), however problems were encountered in the isolation and identification of *C. perfringens* in the present study. The number of viable *Clostridium spp.*, in equine caecal liquor and faeces following intravenous or oral administration of antimicrobial agents, were counted. The perfringens agar base utilized in the present study aids in identification of *C. perfringens* by colonial morphology because the colonies have a black pigmented centre. However, a number of different colonies of similar appearance were seen following incubation of diluted caecal liquor and faecal samples. This was not surprising since there are a huge number of different bacterial species in samples from the gastrointestinal tract. *Bacteroides melaninogenicus* produces a characteristic intracellular or cell-associated water soluble pigment when cultured on blood agar. In addition, a wide range

of organisms have been reported to produce black pigment, due to hydrogen sulphide production, when cultured on cooked meat media containing ferrous sulphate *in vitro*. These include reference strains and laboratory isolates of *Bacteroides spp.*, *E. coli*, *Proteus mirabilis*, *S. typhimurium* and *C. perfringens* (Duerden, 1975). In the present study, *B. melaninogenicus* was not identified using the API system but a number of black colonies from horse blood agar were identified as *B. asaccharolyticus*. Another difficulty was the fact that the limit of detection of the assay of 10^3 cfu was similar to the number of viable *Clostridium spp.* isolated from the samples. The most commonly identified species of the *genus Clostridium* was *C. butyricum*. This organism produced colonies which spread on the agar and may have masked the presence of other *Clostridium spp.*. In addition, following identification of *C. perfringens* by colonial morphology and Gram stain reaction, it was subcultured onto horse blood agar where it produced characteristic α and ß haemolysis following anaerobic incubation. Further identification was by inoculation and incubation of an API anaerobe identification test strip. There were problems in identifying *C. perfringens* due to the reduction of sugars in the test tubes and, despite the addition of bromocresol purple to the tubes where reduction had taken place, it was difficult to differentiate between a positive and a negative reaction. The increase in the number of *Clostridium spp.* recorded ($>10^5$ cfu/ml or /g) was similar to that reported in previous studies (White and Prior, 1982, Wierup and Di Pietro, 1981) and to the number of *Clostridium spp.* isolated following carbohydrate overload (Garner *et al.*, 1978). It is unlikely that a significant increase in the number of one species of clostridia were masked, and it may be that an increase in the total number of viable *Clostridium spp.* is as significant as an increase in the number of one species of this genus.

There were apparent alterations in the number of viable caecal bacteria isolated, particularly following oral administration of antimicrobial agents, in the present study. Oral administration of penicillin G resulted in an increase in the number of viable coliforms, streptococci and *Clostridium spp.* isolated, even although penicillin G is active against Gram positive and some Gram negative bacteria. Similarly, oral administration of ampicillin resulted in an increase in the number of viable coliforms and probable increases in the number of streptococci, lactobacilli and *Clostridium spp.* isolated despite the fact that ampicillin has less activity, compared with penicillin G, *in vitro* against streptococci and *Clostridium spp.* but has greater activity against enterococci. Moreover, there were slight increases in the number of streptococci and lactobacilli isolated following oral administration of amikacin, which is effective mainly against Gram negative bacteria and is of little or no value in an anaerobic environment. There were increases in the number of coliforms, streptococci, lactobacilli and *Clostridium spp.* isolated following the oral administration of oxytetracycline which has a broad spectrum of activity. Thus, despite the differences in the spectrum of activity of the four antimicrobial agents used in the present study, the alterations

in the gastrointestinal microflora appeared to be qualitatively and quantitatively similar. The response was most marked following the oral administration of oxytetracycline, the antimicrobial agent with a spectrum of activity against a wide range of Gram positive and Gram negative bacteria, as well as other microorganisms such as rickettsia. Interestingly clinical signs of depression and anorexia were apparent following oral administration of oxytetracycline to ponies and not following oral administration of the other antimicrobial agents. It would be interesting to investigate the alterations in gastrointestinal microflora following repeated administration of each of the four antimicrobial agents examined in the present study. It would seem probable that the alterations in gastrointestinal would be much more marked following repeated oral administrations, particularly of oxytetracycline, and this may also be the case following repeated intravenous administrations.

In view of the similarities in the response of the bacterial microflora to the different antimicrobial agents, it seems unlikely that the alterations in the number of viable bacteria isolated in the present study were a representation of their *in vitro* susceptibilities. However, an examination of the *in vitro* susceptibility of different gastrointestinal microorganisms to these antimicrobial agents would be required to confirm this.

The number of viable caecal bacteria isolated was similar in different animals and on different occasions. This is quite surprising given the marked variations in the amount of drug measured in the caecal lumen. It is interesting that the peak caecal liquor concentrations of all four of the antimicrobial agents occurred at a similar time after drug administration, namely 1-2 h in pony I and 6-12 h in pony II. Moreover, despite the differences in the dose rates of the four antimicrobial agents, the MRT was similar between occasions and was 1.98-3.92 h in pony I and 6.20-6.75 h in pony II for the penicillins (penicillin G and ampicillin). However, the MRT of amikacin and oxytetracycline, 2.85-5.88 h in pony I and 11.10-19.84 h in pony II, appeared to be longer. This is probably because less amikacin and oxytetracycline were destroyed within the gastrointestinal lumen hence the amount of drug measured in the caecum (AUC) was greater. Although, the volume of caecal contents was estimated the caecal volume varies in relation to feeding and thus the caecal concentrations and hence the AUC would be affected markedly by alterations in the volume of caecal liquor. It would have been useful, given the intestinal transit times of the animals used in the present study, to have taken samples between 12 and 24 h after drug administration.

An increase in the circulating concentrations of endotoxin develops following the disruption of the intestinal mucosal barrier (King and Gerring, 1988, Moore and Morris, 1992). In the present study, the increase in the number of viable coliform bacteria isolated following oral administration of penicillin, ampicillin and oxytetracycline probably would have resulted in an increase in the caecal endotoxin concentrations. Although, all other Gram negative

bacteria (like *Bacteroides spp.*) also produce endotoxin. One could speculate that given a sufficient increase in the number of viable Gram negative bacteria and hence endotoxin (from effete Gram negative bacteria) in the gastrointestinal lumen there would be an increase in peritoneal and consequently plasma endotoxin concentrations. Moore *et al.* (1979) reported an increase in the intra-caecal endotoxin concentrations in the equine following carbohydrate overload, but Garner *et al.* (1978) suggested that there was a reduction in the number of viable enterobacteria due to a drastic reduction in the luminal pH following carbohydrate overload. However, in the ruminant an increase in the number of viable coliform bacteria has been recorded following carbohydrate overload (Allison *et al.*, 1975). The number of viable enterobacteria isolated by Garner *et al.* (1978) was high (10^{10}/ml) prior to carbohydrate overload. Plasma endotoxin concentrations have been measured in horses with colic and in the caecal fluid of horses following carbohydrate overload (King and Gerring, 1988, Moore *et al.*, 1979). It may be useful to measure plasma endotoxin concentrations following oral administration of an antimicrobial agent given the subsequent increase in the number of viable coliforms.

Enterotoxin is of major importance in the development of antimicrobial-associated colitis in man. The major causative organism is *C. difficile*, although other toxin producing bacteria, including staphylococci and *E. coli*, have been isolated from human cases of antimicrobial-associated colitis. *Clostridium difficile* was not isolated in the present study although it has been isolated previously from equines (Jones *et al.*, 1987, Jones *et al.*, 1988). This was probably because *C. difficile* was not present in the environment of the animals used in the present study. Selective isolation of *C. difficile* is sensitive and specific. In man, the diagnosis of *C. difficile* infection is based on the isolation of the organism or the demonstration of toxin production. It would have been useful to examine for toxin production in the present study. However, *C. difficile* has been isolated from an equine source by one group of researchers only, thus the likelihood of isolating *C. difficile* would seem to be low. However, in a hospital environment, where *C. difficile* has been isolated from other sources, toxin detection would be a useful adjunct to the diagnostic armament.

In the present study, serial samples for bacteriological culture were taken every 24 h. However, antimicrobial concentrations were measured in caecal liquor as early as 15 min after drug administration, and peak drug concentrations occurred between 1.5 and 8 h after drug administration. A more frequent sampling regime, particularly in the first 24 to 48 h following antimicrobial administration would provide additional information on the effects of antimicrobial administration on the gastrointestinal microflora. For example, there was an increase in the number of coliforms isolated following oral administration of the two different broad spectrum antimicrobial agents. It may be that this alteration was secondary to a reduction in the number of viable commensal bacteria. It would be interesting to see

whether the increase in the number of viable bacteria isolated was related to drug concentrations and whether the increase, *e. g.* up to 10^9-10^{11} coliforms per ml, reported in the present study was a maximal effect. The 24 hourly sampling regime was probably more appropriate for the faecal samples, given the time taken to reach maximum concentrations of the antimicrobial agents at each site, in the present study. An examination of the number of viable faecal bacteria isolated following oral administration of antimicrobial agents to normal horses would be a means of confirming or refuting the results reported in the present study. In addition, it would be interesting to study counts of caecal and faecal viable bacteria in parallel. This would provide information on how closely the population of microflora in the caecum is reflected by the faecal microbial population. In the present study, different animals kept under similar management conditions had similar numbers of viable bacteria in caecal liquor and in faeces.

Salmonella typhimurium phage type 204c was isolated from one pony on two separate occasions following oral administration of oxytetracycline. A further oral administration of oxytetracycline and repeated sampling failed to re-isolate the organism. It is possible that this was due to immunity to re-infection either due to an immune response following previous exposure to the organism, hence the absence of overt clinical signs on this occasion, or to a rapid protective immune response on the occasion in the present study. A sample of serum from this animal following isolation and identification of *Salmonella typhimurium* phage type 204c agglutinated the organism, which did not exhibit autoagglutination (unpublished data).

Caecal liquor pH was measured as a means of monitoring the large intestinal hydrogen ion concentrations, and the production of SCFAs by the bacteria in the caecal lumen. There was considerable variation in the caecal pH measurements in the present study. Murray (1988a) suggested that in the equine large intestine the pH was maintained between 6.8 and 7.2, and Garner *et al.* (1978) reported control pH values of 7.18±0.14 (mean±SEM) for caecal fluid. However, other studies have reported that the caecal luminal pH was lower than this (pH 6.5-6.9) (Argenzio *et al.*, 1974, Mackie and Wilkins, 1988) and Goodson *et al.* (1988) reported a much wider range of caecal pH of 6.4-7.7 for ponies on a forage diet. This suggests that, in the present study, the majority of the pH measurements were within the normal range. There were some apparent reductions and increases in the caecal pH outside the wider pH ranges and these alterations in the hydrogen ion concentration may reflect alterations in SCFA production in the gastrointestinal lumen. Marked reductions in luminal pH have been associated with diets high in concentrates, and following carbohydrate overload in ruminants and in horses, but in general the reduction in the pH following carbohydrate overload was to a pH of less than 6 (Garner *et al.*, 1978, Moore *et al.*, 1979, Wernery and Wensvoort, 1992). A pH range of pH 6.4-7.7 reported in the present study

represents a wide range of hydrogen ion concentrations of around 2×10^{-8} to 4×10^{-7} M. A pH range of 4 to 6, associated with the development of clinical signs of acidosis, represents a hydrogen ion concentration of 1×10^{-6} to 1×10^{-4} M, and this is up to 10000 times greater than the majority of pH values reported in the present study. It appears that although there were wide variations in hydrogen ion concentrations a marked reduction in caecal liquor pH was prevented by either combining with bicarbonate ions or by exchange with sodium ions across the caecal mucosa. In view of the wide range of normal caecal luminal pH values, an alteration in luminal pH following a single intravenous or oral administration was not a particularly useful indicator of an alteration in SCFA concentrations. There may be more marked alterations in caecal pH following the administration of antimicrobial agents either on a single occasion at a higher dose rate or following repeated dosing, and the measurement of luminal pH has the advantage of being almost instantaneous.

In the present study, SCFA concentrations were measured as a means of monitoring bacterial fermentation in the large intestine. SCFA production can be measured using either an indirect or a direct approach. Indirect methods measure SCFA concentrations or estimate SCFA production from a known amount of substrate metabolized by a known weight of bacteria. Short-chain fatty acids are metabolized and absorbed therefore SCFA concentrations do not represent SCFA production. A direct approach measures SCFA production rates. The technique used in the present study is an indirect means of measuring VFA production, and it assumes that the capacity of VFA absorption can be exceeded, and that changes in VFA absorption and secretion are proportional to changes in VFA production. Other indirect approaches include the use of a suitable fermentation equation, or the dry weight of bacteria excreted in faeces per day, along with the amount of available substrate to calculate theoretical VFA production (Fleming and Arce, 1986, Miller and Wolin, 1979, Smith and Bryant, 1979). However, these methods assume that the only non-gaseous end-products of microbial fermentation are VFAs, and the dietary fibre fermented in the colon is lost as bacterial cells in the faeces, respectively, and this may result in inaccurate estimates. Also, bacterial excretion rates vary with diet, *e. g.* in man it represents an average of 13% of the dry weight of faeces. In ruminants, VFA production rates have been measured directly using complex radioisotope dilution techniques (Bruce *et al.*, 1987). An alternative approach to assessing gastrointestinal fermentation would be to measure SCFA concentrations outside the gastrointestinal tract *e. g.* in plasma or breath. This would be particularly useful since these methods are much less invasive than caecal fistulation and would permit the use of normal animals in studies of gastrointestinal microbial fermentation. In fact this approach has been used in man where there is limited or no access to samples of large intestinal contents. The method of extraction of SCFA from caecal liquor was applied to trial plasma samples and was suitable for the extraction of SCFA from plasma (unpublished data). However, blood from the gastrointestinal tract passes through the liver

and the extensive metabolism of SCFAs in the liver may result in SCFA concentrations in circulating plasma being an unreliable estimate of large intestinal SCFA concentrations.

A novel analytical method was developed to measure NVFA and VFA concentrations simultaneously and this was used to measure SCFA concentrations in equine caecal liquor and faeces. The method used in the present study is simple and rapid in comparison with the other available techniques (Boley and Colwell, 1987, Canale *et al.*, 1984, Guerrant *et al.*, 1982, Holdeman *et al.*, 1977, Mahadevan and Stenroos, 1967). In addition, this method would be applicable to the rapid identification of bacteria *in vitro* (Tabaqchali, 1982) and measurement of SCFA concentrations in plasma (unpublished data). There are some problems associated with the extraction and chromatography of SCFA from biological matrices. The high recovery of the seven SCFA obtained here shows that a solid-phase system is a suitable technique to overcome these problems.

During development of the method described in the present study, different buffering systems (borate and carbonate) were investigated to try and optimize the recovery and selectivity. Attempts to concentrate the effluent from the Sep-pak cartridges, using different buffers and by varying the elution volume, were unsuccessful. The other buffering systems prolonged the solvent front and interfered with the resolution of the lactic acid peak. Extraction with phosphate buffer (0.05 M, pH 7.0) resulted in the best solvent front and maximised the extraction (volume). Unfortunately lactic and acetic acids were eluted in the first 0.5 ml along with some of the dirt contained in the samples. Various attempts were made to clean up the samples by introducing a wash step after loading a sample onto a solid-phase cartridge. Distilled water, buffer (Clark and Lubbs KCl/HCl), and buffer components (KCl, HCl) were tried but this step removed some of the acids (lactic and acetic) and reduced the recoveries of the remaining acids to around 30 %. Filtering, prior to solid-phase extraction, was investigated as a possible clean-up step but this markedly reduced the percentage recoveries. Optimum results were obtained using 3.5 ml of phosphate buffer (0.05 M, pH 7.0) to elute the SCFA from the solid-phase cartridge, followed by filtering the eluted samples through a 0.45 μm cellulose nitrate filter prior to injection into the chromatograph.

The method described in the present study was suitable for measuring seven SCFAs in equine caecal liquor but requires further modification to improve the extraction of SCFAs from faecal samples. There was considerable variation in the faecal SCFA concentrations. This appears have been due to inconsistent extraction of SCFAs from faecal samples although many samples had SCFA concentrations within the normal ranges established for caecal liquor. In addition, faecal lactic acid concentrations appeared to be related to the caecal lactic acid concentrations, and were all within or above the normal range established for

caecal liquor. In man, gastrointestinal VFA concentrations are measured in faecal water or in water from enema samples (Clausen *et al.*, 1991, Fleming *et al.*, 1989, Holtug and Mortensen, 1989, Weaver *et al.*, 1988). Commonly, the SCFAs are extracted from faecal samples using methods such as steam distillation prior to analysis by GLC (Bruce *et al.*, 1987, Clausen *et al.*, 1991). The technique used in the present study was not dissimilar to the one described by Merritt and Smith (1980). These authors passed faeces through wire mesh prior to centrifugation, and extracted the VFAs using *meta*phosphoric acid. It may be that breaking up the sample to reduce the particle size, *e. g.* by seiving, would result in better mixing of the sample with the buffer, and more consistent extraction of faecal SCFA. In addition, a period of mixing or homogenizing of the faecal samples with the buffer may improve the extraction of SCFA from faecal samples. It would be unlikely that lengthening the centrifugation time would improve the recoveries of SCFA from faecal samples.

It was possible to separate the seven SCFA using a standard reverse phase HPLC column although the separation of lactic and acetic acid peaks was poor. A number of solvents containing ion-pairing agents (*e. g.* decylamine, sodium lauryl sulphate) were used to try and improve the separation of lactic and acetic acids but this was unsuccessful. The ion-exchange column used in the present study produced adequate separation and delineation of the seven SCFA peaks. A variety of concentrations of acid were tried in the mobile phase in an attempt to optimize the peak height and shape of each acid and to minimize retention times although these alterations had to be within a reasonably small range in order not to damage the polymer packing material in the column.

There were no endogenous peaks recorded that interfered with the resolution of the seven SCFAs on the chromatogram, although there was an unidentified peak that chromatographed between isovaleric and valeric acids. A similar unidentified peak was reported by Guerrant *et al.* (1982). These researchers examined a large number of biological acids by HPLC but failed to identify this peak. The unidentified peak appeared in standard solutions despite the use of reagents of high purity. In addition, the peak was present in some, but not all, samples at widely varying peak heights. One suggestion was that the unidentified peak represented 2-methylbutyric acid (an isomer of 3-methylbutyric or isovaleric acid). Usually, the unidentified peak was small and did not interfere with the desired peaks so the analytical method was considered to be satisfactory. Separation of the compound using a preparative HPLC would permit identification using an HPLC with a mass spectrometer interface.

The recovery of the SCFAs was calculated using the external standard method. Using an internal standard was considered, however there was some difficulty in choosing a suitable compound. Ideally an internal standard should be similar in retention time to and be extracted in a similar manner to the required compounds but should not interfere with the

desired compounds in any way. This was difficult because of the proximity of the peaks, the relatively long retention times and the possibility of further endogenous peaks in samples. It would have been necessary to choose something that was not naturally occurring (Czerkawski, 1986).

Generally, caecal lactic acid concentrations are low in animals fed a forage diet. In the present study, the median (range) lactic acid concentration of 1.5 mmol/l (0.0-24.4 mmol/l) was similar to the control concentrations reported by Moore *et al.* (1979). There were increases in both caecal liquor and faecal lactic acid concentrations following intravenous and oral administration of antimicrobial agents, although the alterations were most marked following oral administration of antimicrobial agents. The increase in lactic acid concentrations may have been due to an increase in production of lactic acid or to either a decrease in metabolism, *e. g.* to propionate, or a reduction in transport across the intestinal mucosa. If the increase in lactic acid concentration was due to an increase in production of lactic acid then a concomitant reduction in luminal pH would be expected if the intestinal buffering systems were overwhelmed. A reduction in transmucosal transport of lactic acid may be due to inhibition, since butyric acid is a competitive inhibitor of L-lactate uptake (Wolffram *et al.*, 1988). Moreover, lactic acid concentrations may accumulate in the gastrointestinal lumen due to slower absorption of the NVFA compared to the VFA (Umesaki *et al.*, 1979). An increase in lactic acid concentrations may result in an increase in the luminal bicarbonate ion concentrations and a reduction in the absorption of water, which in an extreme situation may lead to the development of diarrhoea. The lactic acid concentrations measured in the present study represented a racemic mixture of both D- and L-lactic acid, and the increase in lactic acid concentrations may have been due to to an increase in D-lactic acid which cannot be metabolized by the animal. Slyter and Rumsey (1991) showed that there were peaks in both D- and L-lactic acid concentrations following carbohydrate overload. It would therefore have been useful to separate D- and L-lactic acid. Racemic mixtures can be separated into their enantiomers using a chiral HPLC column, and this type of separation was attempted unsuccessfully (unpublished data). The short wavelength (210 nm) meant that samples had to be very clean and problems arose with the selection of a solvent that would provide a sufficiently quiet baseline. The increase in gastrointestinal lactic acid concentrations may be related to the concentration of an antimicrobial agent in the gastrointestinal lumen, and to the subsequent effects on the proportions of the different commensal microflora. However, amikacin appeared to have only slight effects on the *genera* of organisms isolated in the present study, despite being present at high concentrations. An increase in lactic acid concentrations may not be related solely to antimicrobial activity in the gastrointestinal tract, and increased lactic acid concentrations have been recorded following carbohydrate overload. The elevated intestinal lactic acid concentrations observed in the present study following administration of

antimicrobial agents were of a similar magnitude (*circa* 42.5 mmol/l) to those recorded by Moore *et al.* (1979) following carbohydrate overload in 2 horses. Lactic acidosis is of clinical importance in the horse and the lactic acid formed by the gastrointestinal microflora is a major source of plasma lactate concentrations (Coffman, 1975a, b). Peak caecal liquor lactic acid concentrations of 7.9-38.7 at 4-12 h, 39.6-51.6 mmol/l at 8-32 h, 7.8-26.4 mmol/l at 4-24 h, and 24.9-50.6 mmol/l at 8-56 h following oral administration of penicillin G, ampicillin, amikacin and oxytetracycline, respectively, were higher, in general, than the peak concentrations of 1.7-10.1 mmol/l at 0.75-12 h, 2.1-5.1 mmol/l at 8-24 h, 3.3-8.9 mmol/l at 4-12 h and 4.5-16.4 mmol/l at 8-72 h following intravenous administration. Interestingly, the highest caecal liquor lactic acid concentrations occurred following oral administration of the broad spectrum antimicrobial agents, ampicillin and oxytetracycline. There was wide variation in the time at which peak lactic acid concentrations occurred despite the similarities in drug disposition in the caecum in each animal on different occasions. It appears that there is a greater risk of developing antimicrobial-associated gastrointestinal disturbances following the administration of broad spectrum antimicrobial agents.

It would have been useful to have measured plasma lactate concentrations in the present study to demonstrate whether the increased intestinal lactate concentrations were reflected in the plasma since lactic acid concentrations in plasma are a good prognostic indicator in horses with acidosis (Gossett *et al.*, 1987). The gastrointestinal tract is by no means the only source of lactic acid in the body. A number of tissues and cell types, including skeletal muscle, red blood cells and hepatocytes, produce lactic acid under a variety of different conditions, including exercise. Moreover, Wolter *et al.* (1983) reported that intra-caecal L-lactate administration caused a high liberation of histamine in the pony caecum but that blood lactate and histamine concentrations were not altered. Thus, the increase in gastrointestinal lactic acid concentrations may only have local effects although this may be of importance in the development of disorders of the gastrointestinal tract, such as colitis. In addition, systemic acidosis may result due to the movement of large quantities of the bicarbonate ion into the gastrointestinal lumen to buffer high concentrations of acid produced there.

In general the total SCFA concentrations in the literature are equivalent to the total VFA concentrations discussed in the present study. The baseline studies demonstrated that there are a wide range of SCFA concentrations in the equine caecum. Rechkemmer *et al.* (1988) reported that in most mammals the SCFA concentrations in the hindgut are around 100 mmol/l despite inter-species differences in production. The ratio (% of total VFA concentration) of acetate, propionate and butyrate was similar in the bovine rumen and in human faeces (65, 20, and 15% and 47.8, 10.8 and 5.3%, respectively) (Miller and Wolin, 1979, Wolin, 1981). In the equine, mean caecal VFA concentrations of 50-60 mmol/l have

been recorded and these were increased up to 80-100 mmol/l following feeding (Argenzio *et al.*, 1974, Argenzio and Stevens, 1975). The ratio of acetic, propionic and butyric acids as a percentage of the total VFA concentration was approximately 70%, 22% and 6% (Argenzio *et al.*, 1974). In ponies, Hintz *et al.* (1971) reported that the ratio of acetic, propionic and butyric acids was 73, 17 and 8% on a hay diet and 59, 25 and 11% on a diet of 1 part hay 5 parts grain. A similar median (range) total VFA concentration of 65.0 mmol/l (24.4-109.2 mmol/l) and a median ratio (range) of 53.1% (27.9-67.0%) acetic acid, 18.7% (4.9-38.5%) propionic acid and 21.3% (8.7-66.7%) butyric acid was measured in the present study. There was a tendency to a higher ratio of butyric acid in the present study.

There were considerable variations in the concentrations of individual VFAs, particularly acetic, propionic and butyric acids, following administration of antimicrobial agents in the present study. These alterations were particularly marked following the oral administration of the antimicrobial agents, and there appeared to be different alterations in VFA concentrations with different antimicrobial agents. Oral administration of penicillin G produced a reduction in the concentrations of butyric and propionic acids and a reduction in the proportion of butyric acid, as a percentage of the total VFA concentrations; ampicillin produced an apparent reduction in the concentration of propionic acid and in the proportion of butyric acid; and amikacin and oxytetracycline, produced little or no alterations in total or individual VFA concentrations or proportions. Interestingly, the two penicillins affected individual VFA concentrations although there were no marked alterations in the total VFA concentrations, whereas amikacin, the antimicrobial agent which produced only slight alterations in numbers of viable bacteria had no marked effects on VFA concentrations or ratios. Oxytetracycline appeared to have the most marked effects on the number of viable bacteria isolated but had little or no effect on VFA concentrations. The antimicrobial agents that affect cell wall synthesis and result in lysis of bacterial cells produced apparent alterations in VFA concentrations whilst the antimicrobial agents which inhibit bacterial protein synthesis, either irreversibly or reversibly, did not affect individual VFA concentrations. Interestingly, the positive metabolic effect of the so-called growth promoting antimicrobial agents, such as the ionophores, is a shift in bacterial fermentation away from acetic and butyric acid and methane production to production of propionic acid. Thus, a relative or actual reduction in the amount of propionic acid may be detrimental to the energy metabolism of the animal.

The alterations in numbers of viable bacteria and lactic acid concentrations are not dissimilar to the alterations in the gastrointestinal tract described following carbohydrate overload in the horse (Garner *et al.*, 1978, Moore *et al.*, 1979). Following carbohydrate overload, there is a predominance of lactic acid producing bacteria, an increase in the lactic acid concentrations with a concomitant reduction in the luminal pH. Lactic acid producing bacteria including

streptococci, lactobacilli and *Clostridium spp.* all increased, particularly following oral administration of the antimicrobial agents, in the present study. In addition, increases in the number of coliforms, lactobacilli, and *Clostridium spp*, isolated have been recorded following carbohydrate overload (Allison *et al.*, 1975, Garner *et al.*, 1978). Slyter and Rumsey (1991) demonstrated that faecal coliform bacteria from horses produced D-lactic acid. However, in cattle there were peaks in both D- and L-lactic acid following carbohydrate overload with the former peak mimicking the peak number of lactobacilli and the latter corresponding to an increase in the number of coliforms. In the present study the reduction in caecal liquor pH was not marked but there were substantial increases in lactic acid concentrations. The changes in the gastrointestinal lumen that follow feeding have been described as a subclinical carbohydrate overload (Clarke *et al.*, 1990b). It is also possible that the alterations in caecal liquor were in fact alterations that occurred within the small intestine and were carried into the caecum. This suggests that alterations reported in the present study may be either a non-specific response of the caecal luminal microflora to an alteration in their environment or a specific response to an increase in the amount of carbohydrate entering the caecum from the ileum, due to carbohydrate-sparing in the upper gastrointestinal tract. Also, it is relevant that the organisms isolated in the present study represent only part of the gastrointestinal microflora which includes many other species of bacteria as well as protozoa.

In the present study, there were no marked alterations in SCFA concentrations following incubation of caecal liquor in the presence of antimicrobial agents *in vitro*. However, there has been extensive work on bacterial fermentation in the rumen and the effects of growth promoting antimicrobial agents, such as the ionophores, *in vitro* which produced similar results to studies *in vivo* (Chalupa *et al.*, 1980, Chirase *et al.*, 1988, Demeyer and Van Nevel, 1987). The difference in results was probably due to the simplicity of the incubation technique used in the present study; more complex continuous culture techniques were used in previous studies. A method for studying the large intestinal microflora of the horse using continuous culture is available (Davies, 1979) and it would be interesting to repeat the studies using continuous culture.

There are only a limited number of antimicrobial agents available for oral administration in the equine. It appears, from the limited *in vitro* studies described in the present study, that feeding may markedly affect the amount of drug available for absorption from the gastrointestinal tract. Many of the agents retained their activity in caecal liquor, where they appeared to be unabsorbed, as demonstrated by the absence of plasma concentrations, but were able to affect the commensal microflora. It has been suggested that food should be withheld for a minimum of 2 h prior to and following oral administration of antimicrobial agents (Watson, 1986). In fact, in many of the published equine pharmacokinetic studies,

food was withheld for varying amounts of time prior to drug administration. In the present study, oral administration of antimicrobial agents was carried out prior to feeding, although food was not withheld deliberately. In addition, some authors have demonstrated that withholding food has little effect on the AUC_{plasma} following oral administration of some antimicrobial agents (Ensink *et al.*, 1992). The apparently enhanced absorption of antimicrobial agents in the foal may be due to differences in diet. In the adult horse, it appears that, due to the binding of antimicrobial agents to hay *in vitro*, withholding of food prior to antimicrobial administration may be beneficial. In addition, there has been some interest in agents which increase the amount of drug in the body (AUC) either by increasing drug absorption from the gastrointestinal tract, by slowing transit time or by reducing drug elimination, by administration of probenecid, a competitive inhibitor of penicillin excretion (Sarasola *et al.*, 1992).

Although a large part of the present study was carried out in two animals with cannulated caecal fistulas, it provides a useful indication of what may happen in the caecum following intravenous or oral administration of an antimicrobial agent. It is interesting to note that the alterations in the caecal microflora appeared to be repeatable in these animals. Further studies utilizing a larger group of animals would be appropriate to confirm the results of the present study. It may be possible, and more relevant, to repeat the studies in normal animals, using slightly different techniques to monitor alterations in large intestinal microbial function.

Conclusions

A suitable therapeutic regime for penicillin G sodium in horses, ponies and donkeys, based on elimination half-lives and typical bacterial susceptibilities, would be intravenous administration at a dose rate of 10 mg/kg bwt every 4 h. Therapeutic plasma concentrations of ampicillin would be maintained by intravenous administration at a dose rate of 10 mg/kg bwt every 6-8 h to horses, ponies and donkeys. Intravenous administration of amikacin at a dose rate of 6 mg/kg bwt every 8 h to horses and 6 h to ponies and donkeys would be suitable for the treatment of susceptible bacterial infections. The long elimination half-life of oxytetracycline means that intravenous administration at a dose rate of 10 mg/kg bwt administered at 48 h in horses and ponies and at 24 h intervals in donkeys would maintain therapeutic concentrations of the drug in plasma. Oral administration of penicillin G, ampicillin, amikacin and oxytetracycline would be unsuitable for systemic antimicrobial therapy in the pony because of their poor systemic availability. A single administration of an antimicrobial agent is a useful means of studying its effects on the gastrointestinal microbial function. There were no clinical side effects following oral administration of penicillin G, ampicillin and amikacin but the ponies exhibited depression and anorexia following oral administration of oxytetracycline. There were alterations in gastrointestinal microflora following antimicrobial administration and these were particularly marked following oral administration and were probably related to the luminal concentrations of the antimicrobial agents. Although there were similarities between the response of the gastrointestinal microflora to different antimicrobial agents, there were quantitative differences which may relate to the mode of action of the antimicrobial agents studied. There were alterations in number of viable bacteria in caecal liquor following administration of penicillin G, ampicillin, amikacin and oxytetracycline. In particular there were increases in the number of coliforms, streptococci, lactobacilli and *Clostridium spp.* isolated with no apparent alterations in the number of *Bacteroides spp.* isolated. Luminal pH was not a particularly useful index for alterations in SCFA concentrations following a single intravenous or oral administration of the antimicrobial agents studied. There were alterations in SCFA concentrations following antimicrobial administration, particularly increases in lactic acid concentrations, although there were decreases in the concentrations of propionic and butyric acids following administration of the penicillins. In general, the response of the gastrointestinal tract to antimicrobial administration was similar to that described following carbohydrate overload. Accordingly, antimicrobial agents should be administered with care in the equine in particular agents which are eliminated to any extent into the gastrointestinal tract. The disruption in microbial metabolism (VFAs) appeared to be greater following administration of the so-called bactericidal antimicrobial agents, and the broad spectrum antimicrobial agents produced greater alterations in lactic acid concentrations than the narrow

spectrum agents. The development of antimicrobial-associated colitis appears to be unrelated to the survival of non-susceptible bacteria, but may be related to the colonization of the gastrointestinal tract by non-susceptible pathogenic bacteria such as *Salmonella spp*. This would suggest that, as in man, a wide range of antimicrobial agents could result in the development of enterocolitis in the equine, particularly where a pathogenic bacterium is present in the environment.

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Appendix A - Penicillin G

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	61.25	63.24	35.26	53.25±9.01
0.083	43.86	NS	30.09	36.98±6.88
0.1	NS	44.20	NS	44.20
0.25	NS	25.71	11.11	18.41±7.30
0.33	22.52	NS	NS	22.52
0.5	16.35	18.61	6.38	13.78±3.76
0.75	10.97	6.95	3.93	7.28±2.04
1	7.51	4.69	2.57	4.92±1.43
1.5	4.31	2.02	1.45	2.59±0.87
2	2.26	1.42	0.68	1.45±0.46
4	0.60	0.07	0.13	0.27±0.17
6	0.16	0.00	0.03	0.06±0.05
8	0.00	0.00	0.01	0.00±0.00
12	0.00	0.00	0.00	0.00±0.00
24	0.00	0.00	0.00	0.00±0.00

Table A1. Plasma concentrations (µg/ml) of penicillin G following intravenous administration to horses

Time (h)	Pony 7	Pony 8	Pony 10	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	74.10	57.98	30.37	54.15±12.77
0.083	49.46	44.88	19.54	37.96±9.30
0.25	23.90	21.91	14.71	20.17±2.79
0.5	9.35	12.61	11.11	11.02±0.94
0.75	5.09	10.93	7.19	7.74±1.71
1	3.15	5.69	5.30	4.71±0.79
1.5	1.24	3.25	3.15	2.55±0.65
2	0.00	1.46	1.72	1.06±0.54
4	0.00	0.22	0.25	0.16±0.08
6	0.00	0.00	0.02	0.01±0.01
8	0.00	0.00	0.00	0.00±0.00
12	0.00	0.00	0.00	0.00±0.00
48	0.00	0.00	0.00	0.00±0.00

Table A2. Plasma concentrations (µg/ml) of penicillin G following intravenous administration to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	NS	64.29	73.45	68.87±4.58
0.083	NS	48.14	58.35	53.25±5.10
0.117	40.93	NS	NS	40.93
0.25	12.02	19.65	27.10	19.59±4.35
0.5	8.20	10.84	11.94	10.33±1.11
0.75	4.84	3.72	6.82	5.13±0.91
1	3.84	2.01	3.86	3.24±0.61
1.5	1.90	0.94	2.08	1.64±0.35
2	1.02	0.47	0.82	0.77±0.16
4	0.21	0.03	0.13	0.12±0.05
6	0.02	0.00	0.00	0.01±0.01
8	0.00	0.00	0.00	0.00±0.00
12	0.00	0.00	0.00	0.00±0.00
48	0.00	0.00	0.00	0.00±0.00

Table A3. Plasma concentrations (μg/ml) of penicillin G following intravenous administration to donkeys

Parameter	Horse 3	Horse 4	Horse 3
t1/2 B2 (min)	8.40	4.62	5.64
t1/2 B1 (min)	51.00	27.06	48.90
Cp0 (μg/ml)	64.53	77.37	46.79
Vc (ml/kg)	154.96	129.24	213.74
AUCobs (μg.h/ml)	30.36	25.45	13.29
AUMCobs (μg.h^2/ml)	24.41	12.93	7.86
AUC (μg.h/ml)	30.85	23.88	12.49
AUMC (μg.h^2/ml)	28.06	12.61	8.91
MRT (min)	48.24	30.48	35.49
Vdarea (ml/kg)	397.77	272.72	941.26
Vdss (ml/kg)	294.84	221.15	570.98
CLb (ml/h.kg)	324.17	418.72	800.49
kel (/h)	2.09	3.24	3.75
k21 (/h)	1.92	4.27	1.67
k12 (/h)	1.74	3.04	2.79

Table A4. Disposition kinetics of penicillin G following intravenous administration to horses

Parameter	Pony 7	Pony 8	Pony 10
t1/2 B2 (min)	3.06	5.10	1.50
t1/2 B1 (min)	18.18	35.46	37.20
Cp0 (μg/ml)	101.30	71.41	50.85
Vc (ml/kg)	98.72	140.04	196.65
AUCobs (μg.h/ml)	20.52	24.94	18.12
AUMCobs (μg.h^2/ml)	6.09	15.27	14.65
AUC (μg.h/ml)	19.60	23.76	17.09
AUMC (μg.h^2/ml)	6.76	15.72	14.25
MRT (min)	17.81	36.74	48.51
Vdarea (ml/kg)	222.83	359.12	523.31
Vdss (ml/kg)	175.78	278.43	487.93
CLb (ml/h.kg)	510.12	420.88	585.07
kel (/h)	5.17	3.01	2.98
k21 (/h)	6.04	3.18	10.32
k12 (/h)	4.71	3.15	15.29

Table A5. Disposition kinetics of penicillin G following intravenous administration to ponies

Parameter	Donkey 14	Donkey 15	Donkey 17
t1/2 B2 (min)	2.64	5.70	6.78
t1/2 B1 (min)	39.96	25.02	33.12
Cp0 (μg/ml)	212.51	78.61	88.15
Vc (ml/kg)	47.06	127.22	113.45
AUCobs (μg.h/ml)	27.21	18.81	24.96
AUMCobs (μg.h^2/ml)	11.89	6.52	10.96
AUC (μg.h/ml)	22.97	18.46	24.31
AUMC (μg.h^2/ml)	10.69	7.17	11.84
MRT (min)	26.22	20.80	26.35
Vdarea (ml/kg)	418.59	325.68	327.48
Vdss (ml/kg)	202.78	210.37	200.44
CLb (ml/h.kg)	435.45	541.72	411.41
kel (/h)	9.25	4.26	3.63
k21 (/h)	1.79	2.86	2.12
k12 (/h)	5.93	1.87	1.62

Table A6. Disposition kinetics of penicillin G following intravenous administration to donkeys

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
coliforms				
0	1.00E+06	3.00E+04	1.00E+03	3.44E+05±3.28E+05
24	1.00E+05	4.00E+04	3.00E+04	5.67E+04±2.19E+04
48	2.00E+07	1.00E+05	1.00E+05	6.73E+06±6.63E+06
streptococci				
0	1.00E+10	3.00E+07	1.00E+09	3.68E+09±3.17E+09
24	1.00E+07	1.00E+07	7.00E+05	6.90E+06±3.10E+06
48	1.00E+10	5.00E+06	3.00E+07	3.35E+09±3.33E+09
lactobacilli				
0	1.00E+05	3.00E+09	2.00E+05	1.00E+09±1.00E+09
24	1.00E+08	1.00E+06	1.00E+07	3.70E+07±3.16E+07
48	1.00E+05	1.00E+04	3.00E+05	1.37E+05±8.57E+04
Bacteroides spp.				
0	1.00E+08	1.20E+10	7.00E+06	4.04E+09±3.98E+09
24	1.00E+09	1.00E+06	1.00E+05	3.34E+08±3.33E+08
48	1.00E+10	1.10E+06	1.20E+07	3.34E+09±3.33E+09
Clostridium spp.				
0	-	-	-	-
24	1.00E+04	-	-	1.00E+04
48	2.10E+04	-	1.00E+04	1.55E+04±4.49E+03

Table A7. Counts of viable bacteria per g faeces following intravenous administration of penicillin G to horses

Time (h)	Pony 7	Pony 8	Pony 10	mean±SEM
coliforms				
0	4.00E+04	5.00E+04	2.00E+05	9.67E+04±5.17E+04
24	1.30E+07	1.00E+10	2.00E+10	1.00E+10±5.77E+09
48	1.00E+07	3.00E+10	2.00E+10	1.67E+10±8.82E+09
streptococci				
0	1.00E+04	3.00E+04	1.00E+06	3.47E+05±3.27E+05
24	1.00E+05	6.00E+04	1.00E+06	3.87E+05±3.07E+05
48	3.00E+04	5.00E+05	2.00E+07	6.84E+06±6.58E+06
lactobacilli				
0	1.00E+05	1.00E+05	5.00E+05	2.33E+05±1.33E+05
24	2.00E+04	4.00E+04	1.00E+06	3.53E+05±3.23E+05
48	3.00E+07	1.00E+05	1.00E+04	1.00E+07±9.98E+06
Bacteroides spp.				
0	9.50E+06	3.10E+06	8.00E+05	4.47E+06±2.60E+06
24	1.00E+06	1.10E+08	1.20E+10	4.04E+09±3.98E+09
48	1.00E+06	1.00E+07	2.20E+08	7.70E+07±7.15E+07
Clostridium spp.				
0	-	-	1.00E+06	1.00E+06
24	-	2.00E+04	2.00E+04	2.00E+04±0.00E+00
48	1.00E+03	2.00E+05	3.00E+05	1.67E+05±8.79E+04

Table A8. Counts of viable bacteria per g faeces following intravenous administration of penicillin G to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
coliforms				
0	2.00E+04	1.00E+06	1.00E+04	3.43E+05±3.28E+05
24	1.00E+06	5.00E+05	1.00E+05	5.33E+05±2.60E+05
48	1.00E+08	2.90E+05	1.00E+05	3.35E+07±3.33E+07
streptococci				
0	2.00E+03	2.00E+05	3.00E+03	6.83E+04±6.58E+04
24	9.00E+05	3.00E+04	2.30E+04	3.18E+05±2.91E+05
48	9.00E+05	1.00E+04	1.00E+05	3.37E+05±2.83E+05
lactobacilli				
0	4.00E+05	5.00E+04	2.00E+04	1.57E+05±1.22E+05
24	3.00E+04	6.00E+04	2.00E+04	3.67E+04±1.20E+04
48	1.00E+06	1.00E+05	4.00E+05	5.00E+05±2.65E+05
Bacteroides spp.				
0	3.00E+05	1.60E+05	3.20E+07	1.08E+07±1.06E+07
24	3.00E+06	2.70E+06	9.00E+05	2.20E+06±6.56E+05
48	1.50E+08	1.20E+07	1.00E+08	8.73E+07±4.03E+07
Clostridium spp.				
0	-	-	-	-
24	-	2.00E+04	-	2.00E+04
48	1.20E+08	1.00E+06	1.00E+05	4.04E+07±3.98E+07

Table A9. Counts of viable bacteria per g faeces following intravenous administration of penicillin G to donkeys

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.0	0.0	0.0	0.0	68.2	0.0	0.0	68.2
24	0.0	2.2	0.0	0.0	40.1	0.0	0.0	42.3
48	0.0	0.0	0.0	0.0	43.4	0.0	0.0	43.4

Table A10. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to horse 3

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.9	1.7	12.3	0.0	50.2	0.0	0.0	64.2
24	0.0	0.0	2.4	0.0	0.0	0.0	0.0	2.4
48	0.0	0.0	2.7	0.0	0.0	0.0	0.0	2.7

Table A11. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to horse 4

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	9.9	0.0	59.4	0.0	0.0	69.3
24	0.0	1.4	4.0	0.0	48.1	0.0	0.0	53.5
48	2.4	2.9	6.5	0.0	55.0	0.0	0.0	64.4

Table A12. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to horse 5

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.5	0.0	1.9	0.0	13.0	0.0	0.0	14.9
48	0.0	0.0	0.0	0.0	11.8	0.0	0.0	11.8

Table A13. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony 7

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	31.8	0.0	0.0	31.8
24	0.0	0.0	0.0	0.0	17.0	0.0	0.0	17.0
48	0.0	0.0	6.9	0.0	45.7	0.0	0.0	52.6

Table A14. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony 8

Time (h)	L	A	P	IB	B	IV	V	Total
0	4.5	3.5	14.7	0.0	90.0	0.0	0.0	108.2
24	2.9	3.5	4.9	0.0	55.6	0.0	0.0	64.0
48	3.7	11.2	8.4	0.0	0.0	0.0	0.0	19.6

Table A15. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony 10

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.7	6.3	17.1	0.0	0.0	0.0	0.0	23.4
24	2.5	5.2	9.9	0.0	0.0	0.0	0.0	15.1
48	3.1	56.6	14.0	0.0	46.4	0.0	0.0	117.0

Table A16. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to donkey 14

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.5	1.9	8.2	0.0	41.2	0.0	0.0	51.3
24	6.2	6.5	9.4	0.0	33.7	0.0	0.0	49.6
48	2.4	2.5	5.9	0.0	42.5	0.0	0.0	50.9

Table A17. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to donkey 15

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.8	5.2	9.5	0.0	56.1	0.0	0.0	70.8
24	1.4	4.5	19.7	0.0	88.0	0.0	0.0	112.2
48	2.8	8.0	7.7	0.0	43.0	0.0	0.0	58.7

Table A18. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to donkey 16

	Horses		Ponies		Donkeys	
Time (h)	L	Total	L	Total	L	Total
0	1.6±0.9	67.2±1.5	1.5±1.5	46.7±32.1	2.7±0.6	48.5±13.8
24	0.0±0.0	32.7±15.5	1.1±0.9	32.0±16.0	3.4±1.5	59.0±28.4
48	0.8±0.8	36.8±18.1	1.2±1.2	28.0±12.5	2.8±0.2	75.5±20.9

Table A19. SCFA concentrations (mmol/kg) (mean±SEM) in faeces following intravenous administration of penicillin G to horses, ponies and donkeys

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
0	21.04	21.59	19.98	20.87±0.47
24	20.97	15.44	18.70	18.37±1.60
48	22.34	21.97	20.01	21.44±0.72

Table A20. Faecal dry matter content (%) following intravenous administration of penicillin G to horses

Time (h)	Pony 7	Pony 8	Pony 10	mean±SEM
0	18.83	17.77	21.19	19.26±1.01
24	17.20	17.80	23.13	19.38±1.88
48	15.86	14.98	19.92	16.92±1.52

Table A21. Faecal dry matter content (%) following intravenous administration of penicillin G to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
0	17.04	20.03	20.02	19.03±1.00
24	24.67	30.46	23.71	26.28±2.11
48	23.61	34.58	23.26	27.15±3.72

Table A22. Faecal dry matter content (%) following intravenous administration of penicillin G to donkeys

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.033	18.66	56.66	53.82	23.36
0.083	12.95	24.31	38.71	16.45
0.25	11.91	16.39	21.98	10.99
0.5	9.77	9.71	11.97	10.21
0.75	9.18	7.23	7.43	8.27
1	8.37	6.23	4.26	6.60
1.5	7.56	3.43	2.06	4.01
2	6.35	2.20	1.19	3.06
4	1.71	0.24	0.14	0.66
6	0.41	0.03	0.02	0.24
8	0.12	0.00	0.00	0.07
12	0.00	0.00	0.00	0.00

Table A23. Plasma concentrations (µg/ml) of penicillin G following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.00	0.00
0.5	0.00	0.60	0.00	0.00
0.75	0.00	0.51	0.00	0.00
1	0.00	0.43	0.00	0.00
1.5	0.00	0.27	0.00	0.00
2	0.00	0.16	0.00	0.00
4	0.00	0.00	0.00	0.00

Table A24. Caecal liquor concentrations (µg/ml) of penicillin G following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
coliforms				
0	7.00E+05	1.00E+08	2.00E+04	1.00E+05
24	5.10E+05	3.00E+05	4.00E+05	1.00E+03
48	1.00E+04	1.00E+04	2.00E+05	1.00E+04
streptococci				
0	4.00E+05	1.00E+07	1.00E+05	1.00E+05
24	3.00E+07	4.00E+06	1.00E+08	1.00E+07
48	1.00E+06	3.00E+05	5.00E+04	2.00E+06
lactobacilli				
0	1.00E+03	1.20E+06	1.00E+04	4.10E+05
24	1.00E+07	1.00E+06	1.00E+07	3.00E+06
48	1.00E+05	1.00E+05	3.00E+04	1.00E+07
Bacteroides spp.				
0	1.10E+11	1.80E+11	2.10E+08	3.90E+11
24	3.10E+10	2.00E+10	4.10E+10	2.10E+10
48	7.00E+08	1.40E+09	1.30E+11	1.10E+11
Clostridium spp.				
0	4.00E+06	1.00E+06	-	4.00E+04
24	3.00E+04	4.00E+06	1.00E+05	1.00E+03
48	1.00E+05	1.00E+05	-	1.10E+05

Table A25. Counts of viable bacteria per ml caecal liquor following intravenous administration of penicillin G to ponies

Time (h)	I1	I2	II1	II2
0	7.2	7.1	7.2	6.7
0.25	7.4	7.0	7.1	6.7
0.5	7.2	6.9	7.2	6.8
0.75	7.2	6.7	7.2	6.7
1	7.1	6.7	7.1	6.8
1.5	7.0	6.9	7.2	6.8
2	7.1	7.2	7.2	6.7
4	6.8	6.7	7.1	6.7
6	7.0	7.0	7.0	7.0
8	6.9	6.7	7.0	6.7
12	6.9	6.8	6.9	6.8
24	6.8	6.9	6.8	6.9
48	7.0	6.9	6.8	7.0

Table A26. Caecal liquor pH following intravenous administration of penicillin G to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.1	11.4	3.0	0.3	9.5	0.8	0.0	25.0
0.25	4.6	16.8	4.5	0.6	10.1	0.6	0.0	32.6
0.5	10.0	15.1	5.1	0.0	7.6	0.5	0.0	28.3
0.75	10.1	20.7	4.6	0.0	11.2	0.7	0.0	37.2
1	7.1	19.5	4.4	0.0	8.3	0.5	0.0	32.7
1.5	1.3	24.2	6.0	0.0	14.4	0.0	0.0	44.6
2	1.5	18.5	5.4	0.0	16.5	0.0	0.0	40.4
4	4.2	25.7	8.3	0.0	29.5	0.0	0.0	63.5
6	3.3	25.7	9.3	0.0	39.0	0.0	0.0	74.0
8	2.2	17.4	7.7	0.0	15.8	0.0	0.0	40.9
12	1.1	34.8	13.0	0.0	26.9	0.0	0.0	74.7
24	2.1	33.4	10.8	0.0	23.9	0.0	0.0	68.1
48	0.0	31.7	9.5	0.0	18.8	0.0	0.0	60.0

Table A27a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of penicillin G to pony I1

Time (h)	A	P	B	P+B
0	45.6	12.0	38.0	50.0
0.25	51.5	13.8	31.0	44.8
0.5	53.4	18.0	26.9	44.9
0.75	55.6	12.4	30.1	42.5
1	59.6	13.5	25.4	38.8
1.5	54.3	13.5	32.3	45.7
2	45.8	13.4	40.8	54.2
4	40.5	13.1	46.5	59.5
6	34.7	12.6	52.7	65.3
8	42.5	18.8	38.6	57.5
12	46.6	17.4	36.0	53.4
24	49.0	15.9	35.1	51.0
48	52.8	15.8	31.3	47.2

Table A27b. VFA concentrations (%) in caecal liquor following intravenous administration of penicillin G to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.7	23.6	7.4	0.0	8.3	7.5	0.0	46.8
0.25	4.0	18.0	6.0	0.0	4.2	0.0	0.0	28.2
0.5	6.2	20.1	6.6	0.0	2.9	0.0	0.0	29.6
0.75	1.6	23.9	7.8	0.0	5.4	0.0	0.0	37.1
1	1.9	24.5	8.0	0.0	6.5	0.0	0.0	39.0
1.5	16.2	16.3	14.1	0.0	3.0	0.0	0.0	33.4
2	17.1	17.1	15.4	0.0	1.8	0.0	0.0	34.3
4	47.4	15.7	21.7	0.0	0.0	0.0	0.0	37.4
6	47.0	17.8	27.4	0.0	13.1	0.0	0.0	58.3
8	59.5	25.2	33.1	0.0	10.0	0.0	0.0	68.3
12	13.8	41.2	16.6	0.0	16.7	0.0	0.0	74.5
24	0.0	24.8	10.2	0.0	11.0	0.0	0.0	46.0
48	11.7	20.2	5.9	0.0	1.1	0.0	0.0	27.2

Table A28a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of penicillin G to pony I2

Time (h)	A	P	B	P+B
0	50.4	15.8	17.7	33.5
0.25	63.8	21.3	14.9	36.2
0.5	67.9	22.3	9.8	32.1
0.75	64.4	21.0	14.6	35.6
1	62.8	20.5	16.7	37.2
1.5	48.8	42.2	9.0	51.2
2	49.9	44.9	5.2	50.1
4	42.0	58.0	0.0	58.0
6	30.5	47.0	22.5	69.5
8	36.9	48.5	14.6	63.1
12	55.3	22.3	22.4	44.7
24	53.9	22.2	23.9	46.1
48	74.3	21.7	4.0	25.7

Table A28b. VFA concentrations (%) in caecal liquor following intravenous administration of penicillin G to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	45.0	15.7	0.7	17.9	0.0	0.0	79.3
0.25	1.8	43.0	12.5	0.5	12.2	2.1	0.0	70.3
0.5	0.0	31.2	9.6	0.6	6.1	2.3	0.0	49.8
0.75	0.0	20.7	6.8	0.0	4.7	2.2	0.0	34.4
1	0.0	19.7	6.5	1.3	4.0	1.4	0.0	32.9
1.5	0.0	25.6	8.5	0.7	9.1	1.8	0.0	45.7
2	1.3	25.5	7.7	0.0	5.5	1.8	0.0	40.5
4	0.0	30.2	8.7	0.6	12.1	2.4	0.0	54.0
6	0.0	33.6	10.8	0.0	14.6	1.4	0.0	60.4
8	0.0	36.2	12.0	0.0	12.4	2.1	0.0	62.7
12	5.0	37.5	15.2	0.0	17.6	1.6	0.0	71.9
24	0.0	46.4	17.8	0.0	24.0	2.0	0.0	90.2
48	1.6	27.2	8.1	0.2	18.4	1.6	0.0	55.5

Table A29a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of penicillin G to pony II1

Time (h)	A	P	B	P+B
0	56.7	19.8	22.6	42.4
0.25	61.2	17.8	17.4	35.1
0.5	62.7	19.3	12.2	31.5
0.75	60.2	19.8	13.7	33.4
1	59.9	19.8	12.2	31.9
1.5	56.0	18.6	19.9	38.5
2	63.0	19.0	13.6	32.6
4	55.9	16.1	22.4	38.5
6	55.6	17.9	24.2	42.1
8	57.7	19.1	19.8	38.9
12	52.2	21.1	24.5	45.6
24	51.4	19.7	26.6	46.3
48	49.0	14.6	33.2	47.7

Table A29b. VFA concentrations (%) in caecal liquor following intravenous administration of penicillin G to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	40.5	11.7	1.3	7.8	0.0	0.0	61.3
0.25	0.0	32.6	13.0	0.0	7.2	0.0	0.0	52.8
0.5	0.0	34.5	13.8	0.0	7.2	1.9	0.0	57.4
0.75	0.0	31.5	10.8	0.0	6.9	0.0	0.0	49.2
1	0.0	34.5	10.0	0.0	10.5	0.0	0.0	55.0
1.5	0.0	38.4	13.3	2.9	8.7	0.0	0.0	63.3
2	0.6	25.6	8.8	1.2	6.4	2.7	0.0	44.7
4	1.7	40.4	13.6	0.0	13.0	0.0	0.0	67.0
6	0.0	36.5	12.3	0.0	14.6	0.0	0.0	63.4
8	1.4	44.0	15.8	0.0	14.6	2.5	0.0	76.9
12	0.0	42.3	16.7	0.9	6.1	6.3	0.0	72.3
24	0.0	40.4	17.6	0.0	9.4	0.0	0.0	67.4
48	2.1	32.2	10.9	0.0	13.6	0.0	0.0	56.7

Table A30a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of penicillin G to pony II2

Time (h)	A	P	B	P+B
0	66.1	19.1	12.7	31.8
0.25	61.7	24.6	13.6	38.3
0.5	60.1	24.0	12.5	36.6
0.75	64.0	22.0	14.0	36.0
1	62.7	18.2	19.1	37.3
1.5	60.7	21.0	13.7	34.8
2	57.3	19.7	14.3	34.0
4	60.3	20.3	19.4	39.7
6	57.6	19.4	23.0	42.4
8	57.2	20.5	19.0	39.5
12	58.5	23.1	8.4	31.5
24	59.9	26.1	13.9	40.1
48	56.8	19.2	24.0	43.2

Table A30b. VFA concentrations (%) in caecal liquor following intravenous administration of penicillin G to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	3.2	0.0	28.0	0.0	0.0	31.2
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	1.0	6.5	3.1	0.0	10.6

Table A31. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	5.4	0.0	0.0	5.4
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table A32. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	15.7	0.0	0.0	15.7
24	0.0	1.6	0.0	0.0	0.0	0.0	0.0	1.6
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table A33. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	4.8	0.0	0.0	0.0	0.0	0.0	4.8
24	0.0	0.0	11.5	0.0	5.1	0.0	0.0	16.6
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table A34. SCFA concentrations (mmol/kg) in faeces following intravenous administration of penicillin G to pony II2

Time (h)	I1	I2	II1	II2
0	23.38	20.25	22.19	20.49
24	22.38	22.00	22.04	24.11
48	23.07	25.55	23.58	24.78

Table A35. Faecal dry matter content (%) following intravenous administration of penicillin G to ponies

	I1			I2		
Time (h)	0	24	48	0	24	48
urea (mmol/l)	5.1	4.9	4.8	4.3	2.9	4.0
sodium (mmol/l)	139	139	139	139	134	134
potassium (mmol/l)	2.7	3.6	2.5	2.9	4.0	3.7
chloride (mmol/l)	93	94	101	99	97	96
calcium (mmol/l)	3.00	2.91	3.21	3.01	3.22	3.05
magnesium (mmol/l)	0.83	0.64	0.74	0.71	0.68	0.82
phosphate (mmol/l)	0.80	0.80	0.74	1.01	0.73	0.88
creatinine (µmol/l)	128	107	120	126	111	134
bilirubin (µmol/l)	17	11	12	14	11	13
SAP (U/l)	488	451	473	441	360	419
AST (U/l)	285	264	279	289	264	304
GGT (U/l)	35	25	27	24	23	26
total protein (g/l)	75	70	79	78	72	80
albumin (g/l)	31	29	29	30	28	32
globulin (g/l)	44	41	46	48	46	48

Table A36. Plasma biochemistry following intravenous administration of penicillin G to pony I

	II1			II2		
Time (h)	0	24	48	0	24	48
urea (mmol/l)	3.5	3.4	4.9	2.9	1.9	2.6
sodium (mmol/l)	141	141	139	140	137	138
potassium (mmol/l)	2.8	3.4	3.0	3.0	4.1	2.4
chloride (mmol/l)	96	96	102	99	100	99
calcium (mmol/l)	2.87	2.79	3.27	3.00	3.17	3.37
magnesium (mmol/l)	0.86	0.64	0.95	0.70	0.66	0.86
phosphate (mmol/l)	0.86	1.00	0.83	1.20	1.16	1.19
creatinine (µmol/l)	108	98	121	94	96	104
bilirubin (µmol/l)	13	10	14	9	8	8
SAP (U/l)	501	441	506	420	359	383
AST (U/l)	318	288	328	342	325	334
GGT (U/l)	41	32	36	31	32	33
total protein (g/l)	73	66	77	76	74	76
albumin (g/l)	35	32	35	32	34	34
globulin (g/l)	38	34	42	44	40	42

Table A37. Plasma biochemistry following intravenous administration of penicillin G to pony II

	I1			I2		
Time (h)	0	24	48	0	24	48
WCC ($x10^9$/l)	6.8	7.9	7.4	6.8	7.0	7.1
RCC ($x10^{12}$/l)	6.83	6.02	5.86	6.15	5.87	6.34
Hb (g/dl)	11.9	10.2	10.2	10.6	10.4	10.4
Hct (l/l)	0.330	0.288	0.276	0.291	0.279	0.300
MCV (fl)	48	48	47	47	48	47
MCH (pg)	17.4	16.9	17.4	17.2	17.7	16.4
MCHC (g/dl)	36.0	35.4	36.9	36.4	37.2	34.6
PLTS (10^9/l)	82	108	105	144	110	139
MPV (fl)	6.2	6.1	6.2	6.3	6.3	6.2
PCT (%)	0.050	0.065	0.065	0.090	14.2	0.086
PDW	17.7	16.3	16.1	14.2	44.9	14.5
Neu (%)	50.0	38.0	45.0	46.0	52.5	53.0
Lym (%)	48.5	56.0	51.0	49.0	1.0	41.6
Mon (%)	1.5	3.0	3.0	4.0	1.0	3.9
Eos (%)	0.0	2.5	0.5	1.0	0.0	1.5
Bas (%)	0.0	0.5	0.5	0.0	0.6	0.0

Table A38. Haematology parameters following intravenous administration of penicillin G to pony I

	II1			II2		
Time (h)	0	24	48	0	24	48
WCC ($x10^9$/l)	7.8	8.0	8.5	6.5	6.9	7.1
RCC ($x10^{12}$/l)	7.82	7.11	7.46	6.97	6.50	6.34
Hb (g/dl)	12.2	11.0	12.0	11.3	11.2	10.0
Hct (l/l)	0.348	0.312	0.324	0.297	0.279	0.273
MCV (fl)	45	44	43	43	43	43
MCH (pg)	15.6	15.4	16.0	16.2	17.2	15.7
MCHC (g/dl)	35.0	35.2	37.0	38.0	40.1	36.6
PLTS (10^9/l)	86	96	99	103	91	121
MPV (fl)	6.6	6.3	6.5	6.5	6.7	6.6
PCT (%)	0.056	0.060	0.064	0.066	0.060	0.079
PDW	15.1	17.4	13.8	13.8	17.9	15.1
Neu (%)	53.0	45.0	65.0	47.0	44.1	57.0
Lym (%)	47.0	52.0	31.0	49.9	51.5	39.0
Mon (%)	0.0	3.0	4.0	2.0	3.0	2.0
Eos (%)	0.0	0.0	0.0	1.1	1.0	2.0
Bas (%)	0.0	0.0	0.0	0.0	0.4	0.0

Table A39. Haematology parameters following intravenous administration of penicillin G to pony II

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.18	0.00	0.08	0.09
0.5	0.19	0.09	0.14	0.16
0.75	0.09	0.04	0.09	0.10
1	0.04	0.02	0.04	0.06
1.5	0.00	0.00	0.00	0.00

Table A40. Plasma concentrations (μg/ml) of penicillin G following oral administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.00	0.00
0.5	39.66	0.00	0.00	0.00
0.75	139.53	60.38	0.00	0.00
1	150.45	89.65	0.00	0.90
1.5	157.12	89.28	0.00	0.88
2	146.11	89.73	0.00	1.18
4	96.06	1.96	98.65	2.17
6	42.49	1.96	118.98	4.96
8	35.58	1.39	89.16	0.92
12	0.00	1.65	0.00	0.61
24	0.00	0.00	0.00	0.00

Table A41. Caecal liquor concentrations (μg/ml) of penicillin G following oral administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
24	0.00	0.65	0.00	0.00
48	0.00	0.00	0.00	0.00

Table A42. Faecal concentrations (μg/g) of penicillin G following oral administration to ponies

Time (h)	I1	I2	II1	II2
coliforms				
0	1.00E+07	1.00E+06	1.00E+06	2.00E+04
24	2.00E+09	1.00E+07	2.00E+09	1.00E+10
48	2.00E+08	2.00E+07	1.00E+10	3.00E+09
72	1.00E+10	1.00E+06	1.00E+10	5.20E+10
96	1.00E+06	1.00E+06	1.00E+06	1.00E+08
168	1.00E+06	1.00E+06	2.00E+04	1.00E+06
streptococci				
0	2.00E+06	3.00E+05	3.00E+06	3.00E+05
24	1.00E+08	2.00E+08	5.00E+07	3.00E+06
48	1.00E+07	1.00E+06	3.00E+10	1.00E+07
72	1.00E+06	5.00E+04	5.00E+10	1.00E+06
96	1.00E+06	5.00E+04	5.00E+06	3.00E+04
168	3.00E+05	4.00E+05	3.00E+05	5.00E+04
lactobacilli				
0	1.00E+06	3.00E+05	7.00E+05	2.00E+05
24	2.00E+08	2.00E+06	6.00E+07	1.00E+06
48	1.00E+07	2.10E+07	1.00E+09	3.00E+06
72	3.00E+05	-	1.00E+07	-
96	1.00E+06	2.10E+05	1.00E+05	1.00E+07
168	3.00E+05	1.00E+06	2.00E+05	2.00E+04
Bacteroides spp.				
0	1.40E+09	1.20E+09	2.40E+11	2.10E+10
24	7.00E+10	1.90E+11	4.00E+10	1.60E+10
48	1.30E+10	-	2.00E+10	5.20E+09
72	1.20E+09	1.10E+08	1.70E+10	1.30E+10
96	6.10E+09	1.20E+10	2.10E+10	8.60E+09
168	1.20E+09	6.60E+08	2.10E+10	1.20E+11
Clostridium spp.				
0	2.10E+05	1.00E+06	1.00E+05	5.00E+05
24	2.20E+08	9.00E+08	2.30E+06	1.00E+05
48	4.80E+07	6.10E+06	2.10E+08	2.00E+06
72	5.00E+05	1.00E+06	1.00E+08	5.30E+08
96	5.00E+04	1.20E+06	5.00E+04	2.00E+07
168	1.00E+06	5.00E+05	5.00E+05	4.00E+04

Table A43. Counts of viable bacteria per ml caecal liquor following oral administration of penicillin G to ponies

Time (h)	I1	I2	II1	II2
0	7.1	7.1	7.0	7.1
0.25	7.1	7.2	7.1	6.9
0.5	7.1	7.1	7.0	7.0
0.75	7.0	7.3	7.1	6.9
1	6.9	7.3	7.2	7.0
1.5	7.1	7.3	7.0	7.1
2	7.3	7.2	7.1	7.0
4	7.7	7.5	7.3	7.2
6	8.3	7.6	7.5	7.3
8	7.9	7.2	7.1	7.2
12	7.4	7.0	6.9	7.1
24	7.5	7.2	7.1	7.0
28	7.0	7.0	7.2	7.1
32	7.0	7.0	7.1	7.0
48	7.1	7.1	6.9	6.8
72	7.1	7.3	6.9	7.2
96	6.8	7.0	6.9	6.9
168	7.1	6.9	7.1	7.0

Table A44. Caecal liquor pH following oral administration of penicillin G to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	19.6	6.6	0.0	4.3	0.0	0.0	30.5
0.25	0.0	19.8	6.5	0.0	4.1	0.0	0.0	30.4
0.5	0.0	20.2	9.0	0.0	6.7	1.4	1.6	38.9
0.75	0.0	24.9	8.7	0.0	10.0	0.0	0.0	43.6
1	0.0	24.7	7.7	0.0	10.2	0.0	0.0	42.6
1.5	0.0	24.9	5.0	0.0	21.6	0.0	0.0	51.5
2	0.0	17.6	7.0	0.0	23.8	0.0	0.0	48.4
4	18.7	14.5	36.4	0.0	14.1	0.0	0.0	65.0
6	22.3	10.9	56.6	0.0	0.0	0.0	0.0	67.5
8	38.7	6.7	46.6	0.0	0.0	0.0	0.0	53.3
12	13.1	8.7	14.7	0.0	0.0	0.0	0.0	23.4
24	2.7	10.2	2.8	0.0	4.5	0.0	0.0	17.5
28	10.1	24.4	4.4	0.0	9.5	0.0	0.0	38.3
32	10.6	28.0	6.9	0.0	6.1	1.6	0.0	42.6
48	0.0	21.7	7.1	0.0	9.4	1.6	0.0	39.8
72	0.0	27.5	8.5	0.0	6.9	2.1	0.0	45.0
96	1.0	30.8	10.2	0.0	5.0	0.0	0.0	46.0
168	0.0	26.1	7.2	1.1	3.8	1.6	0.0	39.8

Table A45a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of penicillin G to pony I1

Time (h)	A	P	B	P+B
0	64.3	21.6	14.1	35.7
0.25	65.1	21.4	13.5	34.9
0.5	51.9	23.1	17.2	40.4
0.75	57.1	20.0	22.9	42.9
1	58.0	18.1	23.9	42.0
1.5	48.3	9.7	41.9	51.7
2	36.4	14.5	49.2	63.6
4	22.3	56.0	21.7	77.7
6	16.1	83.9	0.0	83.9
8	12.6	87.4	0.0	87.4
12	37.2	62.8	0.0	62.8
24	58.3	16.0	25.7	41.7
28	63.7	11.5	24.8	36.3
32	65.7	16.2	14.3	30.5
48	54.5	17.8	23.6	41.5
72	61.1	18.9	15.3	34.2
96	67.0	22.2	10.9	33.0
168	65.6	18.1	9.5	27.6

Table A45b. VFA concentrations (%) in caecal liquor following oral administration of penicillin G to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	26.1	7.2	1.1	3.8	1.6	0.0	39.8
0.25	0.0	16.3	3.7	0.0	6.2	0.0	0.0	26.2
0.5	0.0	15.3	3.6	0.0	4.8	0.7	0.0	24.4
0.75	0.0	12.5	3.1	0.0	6.9	0.0	0.0	22.5
1	0.0	17.1	3.6	0.0	7.5	1.4	0.0	29.6
1.5	0.0	15.1	3.2	0.0	11.3	0.0	0.0	29.6
2	0.0	12.5	3.3	0.0	11.6	0.0	0.0	27.4
4	10.1	6.2	44.4	0.0	13.4	0.0	0.0	64.0
6	0.0	4.3	55.0	0.0	0.0	3.0	0.0	62.3
8	9.4	4.3	60.7	0.0	0.0	0.0	0.0	65.0
12	8.9	12.2	0.0	0.0	3.5	1.9	0.0	17.6
24	5.1	25.1	4.5	0.0	11.1	0.0	0.0	40.7
28	3.1	12.9	1.8	0.0	2.5	0.0	0.0	17.2
32	0.0	25.2	7.1	0.0	7.3	0.0	0.0	39.6
48	0.0	24.5	6.8	0.0	9.5	0.0	1.7	42.5
72	2.8	12.6	4.1	0.0	2.0	1.2	0.0	19.9
96	1.9	24.3	8.9	0.0	12.6	0.0	0.0	45.8
168	1.5	37.5	13.1	0.5	9.3	1.9	0.0	62.3

Table A46a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of penicillin G to pony I2

Time (h)	A	P	B	P+B
0	65.6	18.1	9.5	27.6
0.25	62.2	14.1	23.7	37.8
0.5	62.7	14.8	19.7	34.4
0.75	55.6	13.8	30.7	44.4
1	57.8	12.2	25.3	37.5
1.5	51.0	10.8	38.2	49.0
2	45.6	12.0	42.3	54.4
4	9.7	69.4	20.9	90.3
6	6.9	88.3	0.0	88.3
8	6.6	93.4	0.0	93.4
12	69.3	0.0	19.9	19.9
24	61.7	11.1	27.3	38.3
28	75.0	10.5	14.5	25.0
32	63.6	17.9	18.4	36.4
48	57.6	16.0	22.4	38.4
72	63.3	20.6	10.1	30.7
96	53.1	19.4	27.5	46.9
168	60.2	21.0	14.9	36.0

Table A46b. VFA concentrations (%) in caecal liquor following oral administration of penicillin G to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	35.5	10.2	0.0	7.4	2.2	0.0	55.3
0.25	0.0	29.9	9.4	0.0	5.8	2.4	0.0	47.5
0.5	0.0	30.2	8.5	0.0	6.8	2.6	0.0	48.1
0.75	0.0	26.6	7.6	1.0	7.4	0.0	0.0	42.6
1	0.0	29.0	10.2	1.5	8.4	2.1	0.0	51.2
1.5	0.0	25.9	8.6	0.0	5.7	1.7	0.0	41.9
2	0.0	28.9	10.1	1.1	7.7	2.2	0.0	50.0
4	0.0	24.6	4.6	0.6	7.8	0.0	0.0	37.6
6	0.0	26.6	6.6	0.7	7.5	0.0	0.0	41.4
8	2.4	24.8	4.9	0.7	1.8	1.6	1.1	34.9
12	11.7	23.3	8.5	0.8	0.0	0.0	0.0	32.6
24	9.7	31.2	0.0	0.0	4.8	0.0	0.0	36.0
28	6.2	31.5	4.3	0.0	4.4	3.0	2.2	45.4
32	3.5	23.7	5.9	1.9	3.0	1.2	9.4	45.1
48	8.1	46.2	10.5	0.0	15.9	0.0	0.0	72.6
72	0.0	41.7	12.0	0.0	8.1	2.1	0.0	63.9
96	0.0	43.0	8.8	0.0	9.2	0.0	0.0	61.0
168	0.0	40.7	13.3	0.0	7.3	0.0	0.0	61.3

Table A47a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of penicillin G to pony II1

Time (h)	A	P	B	P+B
0	64.2	18.4	13.4	31.8
0.25	62.9	19.8	12.2	32.0
0.5	62.8	17.7	14.1	31.8
0.75	62.4	17.8	17.4	35.2
1	56.6	19.9	16.4	36.3
1.5	61.8	20.5	13.6	34.1
2	57.8	20.2	15.4	35.6
4	65.4	12.2	20.7	33.0
6	64.3	15.9	18.1	34.1
8	71.1	14.0	5.2	19.2
12	71.5	26.1	0.0	26.1
24	86.7	0.0	13.3	13.3
28	69.4	9.5	9.7	19.2
32	52.5	13.1	6.7	19.7
48	63.6	14.5	21.9	36.4
72	65.3	18.8	12.7	31.5
96	70.5	14.4	15.1	29.5
168	66.4	21.7	11.9	33.6

Table A47b. VFA concentrations (%) in caecal liquor following oral administration of penicillin G to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	40.7	13.3	0.0	7.3	0.0	0.0	61.3
0.25	0.0	26.9	9.7	0.0	5.2	0.0	0.0	41.8
0.5	0.0	37.5	12.3	0.0	7.7	0.0	0.0	57.5
0.75	0.0	38.2	11.8	0.0	7.4	0.0	0.0	57.4
1	0.0	39.7	12.9	0.0	7.8	0.0	0.0	60.4
1.5	0.0	41.1	12.0	0.0	4.8	1.4	0.0	59.3
2	0.0	43.9	14.8	0.0	7.4	0.0	0.0	66.1
4	0.0	19.4	6.2	0.0	4.0	0.0	0.0	29.6
6	0.0	26.6	7.6	0.9	6.9	0.0	3.8	45.8
8	0.0	32.0	6.3	0.0	4.1	0.0	0.0	42.4
12	7.9	30.9	0.0	0.0	5.7	0.0	0.0	36.6
24	7.9	27.3	7.3	0.0	5.3	1.8	0.0	41.7
28	6.2	26.7	10.0	0.0	0.0	0.0	0.0	36.7
32	6.3	29.0	13.2	0.0	7.8	3.0	0.0	53.0
48	0.0	44.9	13.6	0.0	10.4	0.0	0.0	68.9
72	3.4	35.3	15.1	0.0	4.0	2.0	0.0	56.4
96	1.7	41.2	15.7	0.5	7.2	2.5	0.0	67.1
168	0.0	48.1	14.2	2.0	8.2	0.0	0.0	72.5

Table A48a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of penicillin G to pony II2

Time (h)	A	P	B	P+B
0	66.4	21.7	11.9	33.6
0.25	64.4	23.2	12.4	35.6
0.5	65.2	21.4	13.4	34.8
0.75	66.6	20.6	12.9	33.4
1	65.7	21.4	12.9	34.3
1.5	69.3	20.2	8.1	28.3
2	66.4	22.4	11.2	33.6
4	65.5	20.9	13.5	34.5
6	58.1	16.6	15.1	31.7
8	75.5	14.9	9.7	24.5
12	84.4	0.0	15.6	15.6
24	65.5	17.5	12.7	30.2
28	72.8	27.2	0.0	27.2
32	54.7	24.9	14.7	39.6
48	65.2	19.7	15.1	34.8
72	62.6	26.8	7.1	33.9
96	61.4	23.4	10.7	34.1
168	66.3	19.6	11.3	30.9

Table A48b. VFA concentrations (%) in caecal liquor following oral administration of penicillin G to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	6.2	7.9	3.0	0.0	0.0	0.0	0.0	10.9
24	0.0	0.0	12.4	0.0	12.0	0.0	0.0	12.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	6.5	5.0	0.0	0.0	0.0	0.0	0.0	5.0
96	0.0	0.0	0.0	0.0	18.5	0.0	0.0	18.5
168	0.0	0.0	0.0	0.0	18.5	0.0	0.0	18.5

Table A49. SCFA concentrations (mmol/kg) in faeces following oral administration of penicillin G to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	18.5	0.0	0.0	18.5
24	0.0	13.5	3.7	0.0	3.2	0.0	0.0	20.4
48	0.0	14.9	5.8	0.0	6.4	0.0	0.0	27.1
72	0.0	0.0	15.4	0.0	0.0	0.0	0.0	15.4
96	0.0	0.0	2.8	0.0	0.0	0.0	0.0	2.8
168	0.0	0.0	2.8	0.0	2.5	0.0	0.0	5.3

Table A50. SCFA concentrations (mmol/kg) in faeces following oral administration of penicillin G to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	5.3
24	0.0	0.0	0.0	0.0	6.5	0.0	0.0	6.5
48	0.0	29.3	12.2	0.0	12.4	0.0	0.0	53.9
72	0.0	6.7	16.0	0.0	43.2	0.0	0.0	65.9
96	0.0	0.0	7.7	0.0	23.3	0.0	0.0	31.0
168	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table A51. SCFA concentrations (mmol/kg) in faeces following oral administration of penicillin G to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	9.3	0.0	0.0	9.3
48	6.0	9.0	9.5	0.0	0.0	0.0	0.0	18.5
72	0.0	0.0	0.0	0.0	2.5	0.0	0.0	2.5
96	0.0	0.0	13.2	0.0	3.3	0.0	0.0	15.5
168	0.0	0.0	3.3	0.0	21.7	0.0	0.0	25.0

Table A52. SCFA concentrations (mmol/kg) in faeces following oral administration of penicillin G to pony II2

Time (h)	I1	I2	II1	II2
0	23.55	19.29	24.89	22.14
24	24.32	25.28	25.82	17.43
48	23.01	22.32	29.24	18.21
72	22.84	23.80	23.09	22.85
96	21.95	27.62	23.88	24.18
168	19.29	24.78	22.14	22.68

Table A53. Faecal dry matter content (%) following oral administration of penicillin G to ponies

Time (h)	0	24	48	168
urea (mmol/l)	4.5	7.1	5.3	4.1
sodium (mmol/l)	140	137	139	138
potassium (mmol/l)	2.8	2.4	2.8	3.3
chloride (mmol/l)	98	100	98	100
calcium (mmol/l)	3.01	3.09	2.84	2.84
magnesium (mmol/l)	0.58	0.61	0.53	0.56
phosphate (mmol/l)	0.64	0.53	0.56	1.09
creatinine (µmol/l)	137	137	126	98
bilirubin (µmol/l)	13	26	18	13
SAP (U/l)	427	450	421	358
AST (U/l)	277	270	254	273
GGT (U/l)	24	27	26	27
total protein (g/l)	75	76	72	71
albumin (g/l)	30	29	28	31
globulin (g/l)	45	47	44	40

Table A54. Plasma biochemistry following oral administration of penicillin G to pony I1

Time (h)	0	24	48
urea (mmol/l)	4.1	7.3	6.5
sodium (mmol/l)	138	135	138
potassium (mmol/l)	3.3	3.4	2.9
chloride (mmol/l)	100	102	99
calcium (mmol/l)	2.84	2.95	2.90
magnesium (mmol/l)	0.56	0.55	0.68
phosphate (mmol/l)	1.09	0.85	0.88
creatinine (µmol/l)	98	124	117
bilirubin (µmol/l)	13	17	13
SAP (U/l)	358	428	354
AST (U/l)	273	252	226
GGT (U/l)	27	14	19
total protein (g/l)	71	78	71
albumin (g/l)	31	31	29
globulin (g/l)	40	47	42

Table A55. Plasma biochemistry following oral administration of penicillin G to pony I2

Time (h)	0	24	48	168
urea (mmol/l)	3.2	4.3	3.5	6.5
sodium (mmol/l)	141	138	139	136
potassium (mmol/l)	2.9	2.5	2.0	3.4
chloride (mmol/l)	100	101	99	98
calcium (mmol/l)	2.90	3.10	2.81	2.91
magnesium (mmol/l)	0.60	0.73	0.59	0.53
phosphate (mmol/l)	0.70	0.71	0.61	1.11
creatinine (µmol/l)	117	125	109	127
bilirubin (µmol/l)	17	30	25	14
SAP (U/l)	424	502	429	365
AST (U/l)	331	330	299	249
GGT (U/l)	34	37	29	26
total protein (g/l)	78	79	73	71
albumin (g/l)	34	34	32	29
globulin (g/l)	44	45	41	42

Table A56. Plasma biochemistry following oral administration of penicillin G to pony II1

Time (h)	0	24	48
urea (mmol/l)	6.5	5.3	3.9
sodium (mmol/l)	136	137	139
potassium (mmol/l)	3.4	2.5	2.9
chloride (mmol/l)	98	101	99
calcium (mmol/l)	2.91	2.85	3.26
magnesium (mmol/l)	0.53	0.53	0.67
phosphate (mmol/l)	1.11	1.02	1.06
creatinine (µmol/l)	127	96	97
bilirubin (µmol/l)	14	16	10
SAP (U/l)	365	380	372
AST (U/l)	249	271	251
GGT (U/l)	26	23	36
total protein (g/l)	71	76	73
albumin (g/l)	29	32	31
globulin (g/l)	42	44	42

Table A57. Plasma biochemistry following oral administration of penicillin G to pony II2

Time (h)	0	24	48	168
WCC ($x10^9$/l)	7.2	5.5	6.8	7.0
RCC ($x10^{12}$/l)	5.81	5.03	5.81	6.15
Hb (g/dl)	10.3	8.8	10.4	9.5
Hct (l/l)	0.276	0.243	0.276	0.264
MCV (fl)	48	48	48	43
MCH (pg)	17.7	17.4	17.9	15.4
MCHC (g/dl)	37.3	36.2	37.6	35.9
PLTS (10^9/l)	90	126	114	125
MPV (fl)	6.6	5.7	6.1	6.5
PCT (%)	0.059	0.071	0.069	0.081
PDW	15.1	15.7	16.3	16.9
Neu (%)	49.0	66.0	42.1	48.0
Lym (%)	45.0	32.0	53.5	47.0
Mon (%)	1.0	1.4	0.4	3.0
Eos (%)	4.0	0.6	2.5	2.0
Bas (%)	1.0	0.0	1.5	0.0

Table A58. Haematology parameters following oral administration of penicillin G to pony I1

Time (h)	0	24	48
WCC ($x10^9$/l)	7.0	6.8	6.7
RCC ($x10^{12}$/l)	6.15	5.71	5.12
Hb (g/dl)	9.5	10.2	8.7
Hct (l/l)	0.264	0.273	0.246
MCV (fl)	43	48	48
MCH (pg)	15.4	17.8	16.9
MCHC (g/dl)	35.9	37.3	35.3
PLTS (10^9/l)	125	130	124
MPV (fl)	6.5	6.2	6.2
PCT (%)	0.081	0.080	0.076
PDW	16.9	14.5	16.1
Neu (%)	48.0	54.0	50.0
Lym (%)	47.0	42.0	50.0
Mon (%)	3.0	3.0	0.0
Eos (%)	2.0	0.0	0.0
Bas (%)	0.0	1.0	0.0

Table A59. Haematology parameters following oral administration of penicillin G to pony I2

Time (h)	0	24	48	168
WCC ($x10^9$/l)	7.6	6.8	6.4	6.4
RCC ($x10^{12}$/l)	6.69	6.91	7.07	5.68
Hb (g/dl)	11.8	11.0	11.8	10.1
Hct (l/l)	0.285	0.294	0.300	0.267
MCV (fl)	43	43	42	47
MCH (pg)	16.7	15.9	15.8	17.7
MCHC (g/dl)	39.2	37.4	37.3	37.8
PLTS (10^9/l)	93	107	114	119
MPV (fl)	6.6	6.0	6.4	6.0
PCT (%)	0.061	0.064	0.072	0.071
PDW	15.1	16.6	17.1	16.6
Neu (%)	53.0	62.6	46.6	40.9
Lym (%)	39.0	35.0	50.9	57.0
Mon (%)	6.0	1.9	2.0	2.0
Eos (%)	2.0	0.0	0.5	0.0
Bas (%)	0.0	0.5	0.0	0.0

Table A60. Haematology parameters following oral administration of penicilin G to pony II1

Time (h)	0	24	48
WCC ($x10^9$/l)	6.4	5.8	6.4
RCC ($x10^{12}$/l)	5.68	6.34	6.50
Hb (g/dl)	10.1	10.0	10.0
Hct (l/l)	0.267	0.270	0.279
MCV (fl)	47	43	43
MCH (pg)	17.7	15.7	15.3
MCHC (g/dl)	37.8	37.0	35.8
PLTS (10^9/l)	119	127	129
MPV (fl)	6.0	6.6	6.3
PCT (%)	0.071	0.083	0.081
PDW	16.6	15.1	14.2
Neu (%)	40.9	56.0	49.1
Lym (%)	57.0	37.9	47.0
Mon (%)	2.0	5.0	0.9
Eos (%)	0.0	0.0	2.0
Bas (%)	0.0	1.0	0.9

Table A61. Haematology parameters following oral administration of penicillin G to pony II2

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.01	0.02	0.02	0.01±0.00
1	0.59	0.34	0.71	0.63	0.57±0.08
5	5.97	6.70	4.99	4.79	5.61±0.44
10	11.75	9.11	8.81	11.81	10.37±0.82
20	22.70	15.07	22.11	18.26	19.54±1.78
40	27.65	29.85	33.36	39.57	32.61±2.60
80	56.61	61.07	48.28	56.01	55.49±2.66

Table A62a. Concentrations (µg/ml) of penicillin G in caecal liquor following incubation *in vitro* for 3 h

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.00	0.00±0.00
1	0.00	0.03	0.00	0.00	0.01±0.01
5	2.52	2.76	0.00	0.00	1.32±0.76
10	6.00	6.53	0.00	0.00	3.13±1.81
20	19.46	17.69	0.00	1.73	9.72±5.14
40	25.74	29.42	0.17	0.31	13.91±7.93
80	37.90	75.17	0.00	0.00	28.27±18.01

Table A62b. Concentrations (µg/ml) of penicillin G in caecal liquor following incubation *in vitro* for 24 h

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	42.5	11.0	0.0	5.9	1.6	0.0	61.0
0.25	0.3	43.6	14.3	0.3	7.0	0.0	0.0	65.2
1	0.0	38.7	14.3	0.0	6.8	0.0	0.0	59.8
5	0.0	38.1	11.9	0.0	5.9	0.0	0.0	55.9
10	0.0	41.2	13.7	0.0	6.5	0.9	0.2	62.5
20	0.0	45.2	14.9	0.0	8.6	0.7	0.0	69.3
40	0.0	38.5	12.5	0.0	7.0	0.8	0.0	58.8
80	1.5	49.2	14.2	0.0	8.3	0.0	0.0	71.7

Table A63a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 3 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	45.0	13.1	0.0	4.8	0.2	0.0	63.1
0.25	0.0	47.8	14.7	0.0	6.7	0.8	0.8	70.8
1	0.0	43.4	11.2	0.0	4.5	2.4	0.0	61.5
5	0.2	43.9	11.8	0.0	4.6	0.7	1.0	62.1
10	0.0	50.8	12.6	0.0	4.5	2.6	0.0	70.6
20	0.0	47.7	13.6	0.0	5.7	0.2	0.6	67.7
40	0.0	18.4	5.5	0.0	2.9	0.6	0.6	28.0
80	0.0	50.7	16.0	0.0	4.8	0.0	0.0	71.5

Table A63b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 24 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	50.5	13.4	0.0	4.0	0.9	0.6	69.4
0.25	0.0	46.6	12.0	0.0	4.8	0.0	0.0	63.4
1	0.0	44.8	11.7	0.0	6.0	1.0	0.6	64.1
5	0.0	49.1	13.0	0.0	5.7	0.0	0.0	67.9
10	0.0	46.6	12.5	0.0	6.5	0.0	0.0	65.7
20	0.0	53.7	12.2	0.0	4.6	1.8	0.0	72.2
40	0.0	50.9	12.8	0.0	5.9	0.3	0.0	69.8
80	0.0	55.2	17.5	0.0	4.3	0.0	0.0	77.0

Table A64a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 3 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	38.7	11.7	0.0	12.4	0.0	0.0	62.8
0.25	0.0	36.5	11.5	0.0	8.4	1.2	1.8	59.5
1	0.0	41.2	12.1	0.0	7.2	0.0	0.0	60.5
5	0.0	36.8	13.3	0.0	9.4	1.7	0.0	61.3
10	0.0	41.7	10.5	0.0	15.1	0.0	0.0	67.3
20	0.0	34.3	9.0	0.0	8.3	0.0	0.0	51.7
40	0.0	44.6	15.3	0.0	8.8	0.9	1.3	70.8
80	0.3	38.5	12.7	0.7	9.2	1.7	4.3	67.0

Table A64b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 24 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.6	37.0	16.9	0.0	11.1	0.0	0.0	65.0
0.25	1.2	29.8	14.4	0.0	13.0	0.0	0.0	57.3
1	0.4	30.3	19.4	0.0	19.0	0.0	0.0	68.6
5	2.1	34.1	17.4	1.7	8.4	1.6	0.0	63.3
10	1.5	31.4	13.1	0.6	4.1	0.0	0.0	49.2
20	0.7	30.0	12.9	0.0	2.9	0.0	0.0	45.8
40	1.1	28.9	12.0	0.5	2.9	0.9	0.0	45.1
80	0.3	27.8	12.3	0.3	6.0	0.0	0.0	46.5

Table A65a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 3 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.7	29.8	11.5	1.0	5.3	0.8	0.0	48.4
0.25	1.1	14.9	6.4	1.2	4.0	1.2	1.1	28.8
1	0.1	18.5	6.5	0.2	4.6	0.0	0.0	29.8
5	1.2	35.1	13.4	1.2	10.2	0.8	0.0	60.7
10	0.0	35.1	14.9	0.0	12.8	0.0	0.0	62.8
20	1.4	36.6	16.2	1.2	16.3	2.1	0.0	72.4
40	0.0	31.6	10.8	0.5	15.3	0.0	0.0	58.1
80	2.5	31.3	15.7	2.2	19.5	3.5	0.0	72.2

Table A65b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 24 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.3	34.2	13.2	0.7	7.3	1.3	1.1	57.8
0.25	2.0	33.6	13.6	1.7	8.6	2.2	2.9	62.7
1	0.3	35.0	12.9	0.0	7.1	2.0	0.0	57.0
5	2.2	32.7	12.2	1.3	9.7	2.3	2.5	60.7
10	0.9	40.4	14.9	0.3	11.2	1.8	1.5	70.1
20	2.6	34.7	14.0	1.7	14.2	1.1	0.0	65.7
40	1.9	35.3	15.0	1.1	13.6	1.5	2.5	69.0
80	0.3	34.3	15.2	0.0	15.5	0.0	0.0	65.0

Table A66a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 3 h (replicate 4)

Conc. (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.4	34.3	14.9	1.5	10.3	2.1	0.0	63.1
0.25	0.2	35.9	13.9	0.0	6.0	0.7	0.0	56.6
1	2.0	32.2	13.2	1.3	7.0	1.5	0.0	55.2
5	2.4	34.4	13.2	1.5	12.2	1.3	1.2	63.9
10	0.7	35.8	12.8	0.3	14.0	0.0	0.0	62.9
20	2.4	36.0	14.4	1.7	18.0	1.9	2.1	74.1
40	0.3	36.3	14.3	0.0	17.8	0.0	0.0	68.3
80	2.2	31.7	13.7	1.8	15.5	2.3	2.3	67.3

Table A66b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with penicillin G for 24 h (replicate 4)

	3 h		24 h	
Conc (µg/ml)	L	Total	L	Total
0	0.4±0.2	63.3±2.5	0.5±0.3	59.4±3.7
0.25	0.9±0.5	62.2±1.7	0.3±0.3	53.9±8.9
1	0.2±0.1	62.9±2.4	0.5±0.5	51.8±7.5
5	1.1±0.6	62.0±2.5	1.0±0.6	62.0±0.7
10	0.6±0.4	61.9±4.5	0.2±0.2	65.9±1.9
20	0.8±0.6	64.8±6.6	1.0±0.6	67.0±5.2
40	0.8±0.5	60.7±5.8	0.1±0.1	56.3±9.8
80	0.5±0.3	65.1±6.7	1.3±0.6	69.5±1.4

Table A67. SCFA concentrations (mmol/l) (mean±SEM) in caecal liquor following *in vitro* incubation with penicillin G

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
1	0.12	0.11	0.06	0.05	0.09±0.02
2	0.33	0.29	0.17	0.16	0.24±0.04
5	1.01	0.80	0.65	0.73	0.80±0.08
10	2.34	2.26	2.85	2.25	2.43±0.14

Table A68. Concentrations (µg/ml) of penicillin G following incubation *in vitro* at pH 1.9 for 1 h

Appendix B - Ampicillin

Time (h)	Horse 1	Horse 2	Horse 3	Horse 4	Horse 6	mean±SEM
0	0.00	0.00	0.00	0.00	0.00	0.00±0.00
0.033	59.49	61.79	52.19	NS	NS	57.82±2.89
0.083	50.22	54.27	43.41	NS	NS	49.30±3.17
0.25	37.84	38.01	34.71	18.99	25.31	30.97±3.78
0.5	29.58	25.94	27.18	14.90	18.81	23.28±2.76
0.75	21.38	18.26	16.03	13.66	13.84	16.63±1.45
1	14.39	14.11	7.01	11.51	12.17	11.84±1.33
1.5	8.35	6.95	5.33	8.75	7.92	7.46±0.61
2	4.16	3.39	3.21	8.06	6.02	4.97±0.92
4	1.27	0.39	0.41	3.76	2.03	1.57±0.63
6	NS	NS	NS	2.17	1.07	1.62±0.55
8	0.13	0.03	0.03	1.55	0.61	0.47±0.29
12	0.00	0.00	0.00	NS	NS	0.00±0.00
24	0.00	0.00	0.00	0.00	0.00	0.00±0.00

Table B1. Plasma concentrations (μg/ml) of ampicillin following intravenous administration to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	Pony 11	Pony 12	mean±SEM
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
0.033	NS	NS	NS	50.76	41.93	NS	46.35±4.41
0.083	NS	NS	NS	31.76	40.07	53.48	41.77±6.33
0.25	54.02	44.99	37.57	25.35	35.19	29.17	37.72±4.29
0.5	29.76	30.69	21.25	13.76	21.30	23.98	23.46±2.56
0.75	18.16	29.12	12.50	10.06	14.93	14.70	16.58±2.74
1	13.03	19.16	6.97	7.04	14.44	8.22	11.48±2.01
1.5	7.44	15.17	3.41	3.88	7.39	4.60	4.21±1.39
2	3.85	10.97	1.52	2.76	3.45	2.68	1.20±0.49
4	0.32	3.52	1.36	0.85	0.62	0.53	0.57±0.45
6	0.00	1.89	0.39	NS	0.00	NS	0.15±0.15
8	0.00	0.90	0.00	0.00	0.00	0.00	0.00±0.00
12	NS	NS	NS	0.00	0.00	0.00	0.00±0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00

Table B2. Plasma concentrations (μg/ml) of ampicillin following intravenous administration to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	62.68	21.82	56.85	47.12±12.76
0.083	49.24	17.42	50.56	39.07±10.83
0.25	21.47	9.96	28.94	20.12±5.52
0.5	14.32	7.88	14.87	12.36±2.24
0.75	12.32	6.28	10.45	9.68±1.79
1	6.43	6.27	7.53	6.74±0.40
1.5	2.55	3.89	3.97	3.47±0.46
2	1.32	3.03	2.93	2.43±0.55
4	0.13	0.90	0.47	0.50±0.22
6	NS	0.25	NS	0.25
8	0.00	0.09	0.00	0.03±0.03
12	0.00	0.00	0.00	0.00±0.00
24	0.00	0.00	0.00	0.00±0.00

Table B3. Plasma concentrations (μg/ml) of ampicillin following intravenous administration to donkeys

Parameter	Horse 1	Horse 2	Horse 3	Horse 4	Horse 6
t1/2 B2 (min)	22.56	6.24	19.92	24.06	24.00
t1/2 B1 (min)	72.12	34.08	42.66	148.74	110.76
Cp0 (μg/ml)	59.70	67.92	53.16	23.01	34.07
Vc (ml/kg)	167.52	147.23	188.10	434.58	293.52
AUCobs (μg.h/ml)	48.63	42.73	36.33	59.34	46.49
AUMCobs (μg.h^2/ml)	51.71	32.72	28.28	205.08	105.80
AUC (μg.h/ml)	47.89	41.27	36.97	52.14	42.56
AUMC (μg.h^2/ml)	52.87	31.67	29.51	169.01	85.59
MRT (min)	63.80	45.94	46.71	207.36	136.55
Vdarea (ml/kg)	362.19	198.66	277.61	685.97	625.82
Vdss (ml/kg)	230.51	185.96	215.90	621.70	472.47
CLb (ml/h.kg)	208.80	242.31	270.47	191.80	234.95
kel (/h)	1.25	1.65	1.44	0.44	0.80
k21 (/h)	0.85	4.94	1.41	1.09	0.81
k12 (/h)	0.32	1.30	0.21	0.47	0.50

Table B4. Disposition kinetics of ampicillin following intravenous administration to horses

Parameter	Pony 7	Pony 8	Pony 9	Pony 11	Pony 12	Pony 13
t1/2 B2 (min)	7.38	16.62	15.48	2.40	25.20	5.28
t1/2 B1 (min)	33.48	96.72	122.28	48.78	48.42	39.84
Cp0 (μg/ml)	128.74	68.64	72.21	75.63	43.55	81.31
Vc (ml/kg)	77.67	145.69	138.49	132.23	299.63	122.98
AUCobs (μg.h/ml)	55.64	82.18	36.57	28.63	37.86	36.14
AUMCobs (μg.h^2/ml)	37.17	175.88	34.14	27.98	32.28	27.71
AUC (μg.h/ml)	32.79	76.10	36.19	26.33	37.14	33.75
AUMC (μg.h^2/ml)	32.57	143.64	40.86	27.24	35.02	26.71
MRT (min)	40.08	128.41	56.01	58.64	51.16	46.00
Vdarea (ml/kg)	155.28	305.76	812.49	445.56	313.78	284.11
Vdss (ml/kg)	121.15	248.06	311.99	392.97	253.94	234.54
CLb (ml/h.kg)	192.86	131.42	276.31	379.86	269.29	296.31
kel (/h)	2.48	0.90	2.00	2.87	1.17	2.41
k21 (/h)	2.82	1.19	0.46	5.08	1.21	3.39
k12 (/h)	1.58	0.84	0.57	10.02	0.13	3.08

Table B5. Disposition kinetics of ampicillin following intravenous administration to ponies

Parameter	Donkey 15	Donkey 16	Donkey 17
t1/2 B2 (min)	5.40	4.62	9.06
t1/2 B1 (min)	30.06	66.12	46.08
Cp0 (μg/ml)	74.60	25.98	64.43
Vc (ml/kg)	134.06	384.95	155.20
AUCobs (μg.h/ml)	26.05	19.23	31.16
AUMCobs (μg.h^2/ml)	14.98	25.93	24.67
AUC (μg.h/ml)	25.81	18.93	29.36
AUMC (μg.h^2/ml)	14.99	27.62	23.40
MRT (min)	34.50	80.90	47.50
Vdarea (ml/kg)	280.17	840.20	377.23
Vdss (ml/kg)	225.08	771.08	271.32
CLb (ml/h.kg)	387.49	528.37	340.55
kel (/h)	2.89	1.37	2.19
k21 (/h)	3.67	4.14	1.89
k12 (/h)	2.49	4.16	1.42

Table B6. Disposition kinetics of ampicillin following intravenous administration to donkeys

Time (h)	Horse 3	Horse 4	Horse 6	mean±SEM
coliforms				
0	-	1.00E+03	1.00E+05	5.05E+04±4.04E+04
24	3.00E+04	1.00E+05	4.00E+06	1.38E+06±1.31E+06
48	-	1.00E+04	1.00E+10	5.00E+09±4.08E+09
streptococci				
0	1.00E+07	5.00E+06	4.00E+06	6.33E+06±1.86E+06
24	1.00E+06	1.00E+07	1.00E+07	7.00E+06±3.00E+06
48	2.00E+06	3.00E+06	9.00E+08	3.02E+08±2.99E+08
lactobacilli				
0	1.00E+06	1.00E+05	1.00E+06	7.00E+05±3.00E+05
24	6.00E+04	1.00E+07	3.00E+07	1.34E+07±8.80E+06
48	1.00E+06	1.00E+08	1.00E+08	6.70E+07±3.30E+07
Bacteroides spp.				
0	1.70E+07	1.00E+09	1.00E+06	3.39E+08±3.30E+08
24	5.00E+06	1.30E+07	5.30E+07	2.37E+07±1.48E+07
48	2.30E+08	1.10E+08	3.10E+08	2.17E+08±5.81E+07
Clostridium spp.				
0	-	-	2.00E+04	2.00E+04
24	-	3.00E+03	1.00E+05	5.15E+04±3.96E+04
48	1.00E+03	2.00E+03	1.00E+05	3.43E+04±3.28E+04

Table B7. Counts of viable bacteria per g faeces following intravenous administration of ampicillin to horses

Time (h)	Pony 7	Pony 8	Pony 9	mean±SEM
coliforms				
0	1.00E+04	1.00E+05	1.00E+04	4.00E+04±3.00E+04
24	3.00E+07	1.00E+08	1.00E+08	7.67E+07±2.33E+07
48	1.90E+07	1.00E+07	2.00E+08	7.63E+07±6.19E+07
streptococci				
0	1.00E+05	1.00E+05	4.00E+05	2.00E+05±1.00E+05
24	2.00E+08	1.00E+05	1.00E+06	6.70E+07±6.65E+07
48	2.00E+07	1.00E+08	5.00E+06	4.17E+07±2.95E+07
lactobacilli				
0	3.00E+06	2.00E+04	1.00E+06	1.34E+06±8.77E+05
24	1.40E+07	1.00E+06	7.00E+05	5.23E+07±4.38E+07
48	2.00E+07	3.00E+05	2.00E+06	7.43E+06±6.30E+06
Bacteroides spp.				
0	2.00E+07	8.00E+06	1.00E+06	9.67E+06±5.55E+06
24	1.10E+09	1.40E+07	3.20E+07	3.82E+08±3.59E+08
48	1.20E+08	1.40E+10	3.10E+08	4.81E+09±4.60E+09
Clostridium spp.				
0	3.00E+03	2.00E+03	-	2.50E+03±4.08E+02
24	1.00E+06	6.00E+03	-	5.03E+05±4.06E+05
48	2.00E+05	3.00E+04	2.10E+06	7.77E+05±6.63E+05

Table B8. Counts of viable bacteria per g faeces following intravenous administration of ampicillin to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
coliforms				
0	1.00E+05	1.00E+03	1.00E+04	3.70E+04±3.16E+04
24	1.00E+06	1.00E+05	2.00E+04	3.73E+05±3.14E+05
48	2.00E+05	1.00E+08	1.00E+06	3.37E+07±3.31E+07
streptococci				
0	1.00E+08	1.00E+09	1.00E+08	4.00E+08±3.00E+08
24	1.00E+08	2.00E+06	3.00E+06	3.50E+07±3.25E+07
48	1.00E+08	1.00E+08	5.00E+06	6.83E+07±3.17E+07
lactobacilli				
0	2.00E+07	1.00E+07	5.00E+06	1.17E+07±4.41E+06
24	1.00E+06	2.00E+05	2.00E+04	4.07E+05±3.01E+05
48	2.00E+08	1.00E+06	2.00E+09	7.34E+08±6.36E+08
Bacteroides spp.				
0	1.00E+08	3.00E+06	4.00E+06	3.57E+07±3.22E+07
24	9.00E+06	1.00E+07	1.10E+06	6.70E+06±2.81E+06
48	1.20E+07	2.10E+09	1.30E+09	1.14E+09±6.08E+08
Clostridium spp.				
0	2.00E+05	-	1.00E+03	1.01E+05±8.12E+04
24	1.00E+04	-	-	1.00E+04
48	-	2.10E+04	1.00E+04	1.55E+04±4.49E+03

Table B9. Counts of viable bacteria per g faeces following intravenous administration of ampicillin to donkeys

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	3.9
24	0.0	1.4	2.0	0.0	1.6	0.0	0.0	5.0
48	4.6	0.0	2.6	0.0	4.3	0.0	0.0	6.9

Table B10. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to horse 3

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	9.7	0.0	0.0	9.7
24	0.0	0.0	4.7	0.0	0.0	0.0	0.0	4.7
48	0.0	0.0	0.0	0.0	3.2	0.0	0.0	3.2

Table B11. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to horse 4

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	1.7	0.0	2.3	4.0	0.0	0.0	8.0
24	0.0	0.0	3.6	0.0	0.0	0.0	0.0	3.6
48	0.0	13.9	7.4	0.0	16.1	3.2	0.0	40.6

Table B12. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to horse 6

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	11.3	5.0	0.0	15.5	0.0	0.0	31.8
24	0.0	10.4	4.9	0.0	6.6	2.4	2.8	27.1
48	0.0	5.6	8.0	0.0	0.0	0.0	0.0	13.6

Table B13. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony 7

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	7.9	4.7	0.0	18.0	0.0	0.0	30.6
24	0.0	3.0	0.0	0.0	0.0	0.0	0.0	3.0
48	0.0	7.8	0.0	0.0	12.5	0.0	0.0	20.3

Table B14. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony 8

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.1	11.6	7.7	0.0	19.4	0.0	0.0	38.7
24	0.0	0.0	0.0	0.0	3.4	0.0	0.0	3.4
48	0.0	6.2	4.4	0.0	20.9	0.0	0.0	31.5

Table B15. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony 9

Time (h)	L	A	P	IB	B	IV	V	Total
0	8.3	27.8	12.8	0.0	23.7	0.0	0.0	64.3
24	6.3	24.1	4.1	0.0	18.9	3.4	0.0	50.5
48	8.4	3.1	10.0	0.0	98.7	0.0	0.0	111.8

Table B16. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to donkey 15

Time (h)	L	A	P	IB	B	IV	V	Total
0	7.5	24.6	8.8	0.0	35.0	2.0	0.0	70.4
24	0.0	3.0	0.0	0.0	19.6	0.0	0.0	22.6
48	0.0	19.6	5.5	0.0	22.0	0.0	0.0	47.1

Table B17. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to donkey 16

Time (h)	L	A	P	IB	B	IV	V	Total
0	7.7	25.0	7.7	0.0	42.9	0.0	0.0	75.6
24	0.0	6.6	0.0	0.0	8.8	1.5	0.0	16.9
48	0.0	13.5	5.5	0.0	17.9	0.0	0.0	36.9

Table B18. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to donkey 17

	Horses		Ponies		Donkeys	
Time (h)	L	Total	L	Total	L	Total
0	0.0±0.0	7.2±1.7	0.0±0.0	33.7±2.5	7.8±0.2	70.1±3.3
24	0.0±0.0	4.4±0.4	0.0±0.0	11.2±8.0	2.1±2.1	30.0±10.4
48	1.5±1.5	16.9±11.9	1.0±1.0	21.8±5.2	2.8±2.8	65.3±23.5

Table B19. SCFA concentrations (mmol/kg) (mean±SEM) in faeces following intravenous administration of ampicillin to horses, ponies and donkeys

Time (h)	Horse 3	Horse 4	Horse 6	mean±SEM
0	17.22	17.79	23.41	19.47±1.98
24	18.02	16.85	15.94	16.94±0.60
48	19.57	18.32	16.62	18.17±0.85

Table B20. Faecal dry matter content (%) following intravenous administration of ampicillin to horses

Time (h)	Pony 7	Pony 8	Pony 9	mean±SEM
0	17.09	17.48	17.16	17.24±0.12
24	20.59	19.23	20.22	20.01±0.41
48	19.74	17.19	19.09	18.67±0.77

Table B21. Faecal dry matter content (%) following intravenous administration of ampicillin to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
0	19.15	28.09	12.64	19.96±4.48
24	21.26	26.56	17.97	21.93±2.50
48	21.93	18.19	21.06	20.39±1.13

Table B22. Faecal dry matter content (%) following intravenous administration of ampicillin to donkeys

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	25.08	16.42	19.47	15.74
0.5	17.95	14.69	17.25	13.59
0.75	15.43	14.19	15.17	13.25
1	13.09	13.98	14.65	11.91
1.5	9.51	10.91	11.43	9.48
2	7.01	9.40	9.13	8.05
4	2.37	2.60	4.25	3.44
6	0.81	1.39	1.97	2.75
8	0.19	0.43	0.77	1.59
12	0.00	0.00	0.00	0.37
24	0.00	0.00	0.00	0.00

Table B23. Plasma concentrations (µg/ml) of ampicillin following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	1.13	0.00	0.00
0.5	0.00	0.78	0.00	0.00
0.75	0.00	0.75	0.00	0.00
1	0.58	0.46	0.00	0.00
1.5	2.26	0.00	0.00	0.00
2	1.78	0.00	0.00	0.00
4	0.00	0.58	0.00	0.00
6	0.00	0.29	0.00	0.00
8	0.00	0.00	0.00	0.00

Table B24. Caecal liquor concentrations (µg/ml) of ampicillin following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
coliforms				
0	6.00E+05	3.00E+05	1.10E+05	7.00E+03
24	3.20E+06	3.00E+06	2.00E+06	2.00E+05
48	3.00E+05	3.00E+05	2.00E+06	3.00E+03
streptococci				
0	1.40E+06	4.00E+05	1.00E+05	8.00E+05
24	1.00E+08	9.00E+05	3.00E+05	3.00E+06
48	2.00E+06	1.00E+05	2.00E+08	1.00E+05
lactobacilli				
0	1.00E+07	1.00E+06	1.00E+07	1.00E+07
24	1.00E+07	6.00E+06	6.00E+05	5.00E+05
48	3.00E+06	1.00E+05	1.00E+07	5.00E+05
Bacteroides spp.				
0	6.00E+09	2.00E+09	5.00E+10	2.00E+10
24	1.00E+10	2.00E+08	1.50E+07	4.30E+07
48	1.10E+08	5.00E+07	5.30E+9	3.20E+10
Clostridium spp.				
0	-	3.00E+04	-	-
24	2.00E+04	1.90E+06	-	1.00E+04
48	1.20E+05	1.00E+05	5.00E+04	1.20E+05

Table B25. Counts of viable bacteria per ml caecal liquor following intravenous administration of ampicillin to ponies

Time (h)	I1	I2	II1	II2
0	6.8	7.2	6.6	6.7
0.25	7.5	7.8	6.7	6.4
0.5	7.2	7.5	6.8	6.5
0.75	7.5	7.8	7.1	6.4
1	7.4	7.6	6.8	6.4
1.5	7.5	7.7	6.8	6.5
2	7.5	7.7	7.3	6.4
4	7.5	7.2	6.9	6.6
6	7.3	7.1	7.2	6.7
8	7.0	7.3	7.1	6.9
12	6.8	7.0	6.7	6.8
24	7.1	6.9	7.1	6.7
48	7.2	7.0	7.5	6.4

Table B26. Caecal liquor pH following intravenous administration of ampicillin to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	40.2	13.7	0.0	12.9	0.0	0.0	66.8
0.25	0.0	24.5	5.9	0.0	0.0	0.0	0.0	30.4
0.5	0.0	22.6	7.3	0.0	6.4	0.0	0.0	36.3
0.75	0.0	22.7	6.2	0.0	7.0	0.0	0.0	35.9
1	0.0	23.5	6.1	0.0	5.9	0.0	0.0	35.5
1.5	0.0	26.5	6.8	0.0	7.5	1.0	1.0	42.8
2	1.2	23.8	7.2	0.0	8.4	0.0	0.0	39.4
4	19.5	16.3	6.6	0.0	28.0	0.0	0.0	50.9
6	24.7	11.0	17.1	0.0	13.5	0.0	0.0	41.6
8	43.6	8.8	22.4	0.0	8.2	0.0	0.0	39.4
12	33.4	13.6	9.8	0.0	11.9	0.0	0.0	35.3
24	10.1	8.4	4.1	0.0	0.0	0.0	0.0	12.5
48	2.2	33.2	14.7	0.0	24.8	0.0	0.0	72.7

Table B27a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of ampicillin to pony I1

Time (h)	A	P	B	P+B
0	60.2	20.5	19.3	39.8
0.25	80.6	19.4	0.0	19.4
0.5	62.3	20.1	17.6	37.7
0.75	63.2	17.3	19.5	36.8
1	66.2	17.2	16.6	33.8
1.5	61.9	15.9	17.5	33.4
2	60.4	18.3	21.3	39.6
4	32.0	13.0	55.0	68.0
6	26.4	41.1	32.5	73.6
8	22.3	56.9	20.8	77.7
12	38.5	27.8	33.7	61.5
24	67.2	32.8	0.0	32.8
48	45.7	20.2	34.1	54.3

Table B27b. VFA concentrations (%) in caecal liquor following intravenous administration of ampicillin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	20.9	4.8	1.3	9.8	1.2	0.0	38.0
0.25	0.0	18.1	3.7	0.0	7.5	0.0	0.0	29.3
0.5	0.0	17.4	2.8	0.0	8.9	0.0	0.0	29.1
0.75	0.0	20.0	4.7	0.0	9.2	1.1	0.0	35.0
1	0.0	19.4	4.1	0.6	7.0	1.8	0.0	32.9
1.5	0.0	17.9	3.4	0.0	8.3	2.6	0.0	32.2
2	0.0	17.9	3.4	0.0	8.3	2.6	0.0	32.2
4	1.9	23.2	7.9	0.0	27.1	1.2	0.0	59.4
6	4.8	20.8	5.2	0.0	19.4	0.0	0.0	45.4
8	9.5	19.7	6.1	0.0	28.5	0.0	0.0	54.3
12	19.9	20.0	6.3	0.0	20.2	0.0	0.0	46.5
24	44.4	16.5	9.1	0.0	20.8	0.0	5.7	52.1
48	0.0	54.5	24.6	0.0	15.1	0.0	0.0	94.2

Table B28a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of ampicillin to pony I2

Time (h)	A	P	B	P+B
0	55.0	12.6	25.8	38.4
0.25	61.8	12.6	25.6	38.2
0.5	59.8	9.6	30.6	40.2
0.75	57.1	13.4	26.3	39.7
1	59.0	12.5	21.3	33.7
1.5	55.6	10.6	25.8	36.3
2	55.6	10.6	25.8	36.3
4	39.1	13.3	45.6	58.9
6	45.8	11.5	42.7	54.2
8	36.3	11.2	52.5	63.7
12	43.0	13.5	43.4	37.0
24	31.7	17.5	39.9	57.4
48	57.9	26.1	16.0	42.1

Table B28b. VFA concentrations (%) in caecal liquor following intravenous administration of ampicillin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	27.9	7.0	0.0	6.9	0.0	0.0	41.8
0.25	0.0	35.9	9.9	0.0	10.0	0.0	0.0	55.8
0.5	0.0	27.1	6.1	0.0	6.5	0.0	0.0	39.7
0.75	0.0	30.4	9.2	0.0	8.9	0.0	0.0	48.5
1	0.0	27.7	7.3	0.0	6.7	0.0	0.0	41.7
1.5	0.0	25.1	6.7	0.7	5.0	0.0	0.0	37.5
2	0.0	14.0	4.1	0.0	4.4	0.0	0.0	22.5
4	0.0	28.1	7.0	0.0	7.5	0.7	0.0	43.3
6	0.0	28.8	8.2	0.0	8.5	0.0	0.0	45.5
8	5.1	37.7	13.5	0.0	22.0	0.0	0.0	73.2
12	3.8	25.7	16.6	0.0	7.0	0.0	0.0	49.3
24	5.1	18.3	10.7	0.0	4.0	0.0	0.0	33.0
48	0.0	15.9	6.9	0.0	3.7	3.4	0.0	29.9

Table B29a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of ampicillin to pony II1

Time (h)	A	P	B	P+B
0	66.7	16.7	16.5	33.3
0.25	64.3	17.7	17.9	35.7
0.5	68.3	15.4	16.4	31.7
0.75	62.7	19.0	18.4	37.3
1	66.4	17.5	16.1	33.6
1.5	66.9	17.9	13.3	31.2
2	62.2	18.2	19.6	37.8
4	64.9	16.2	17.3	33.5
6	63.3	18.0	18.7	36.7
8	51.5	18.4	30.1	48.5
12	52.1	33.7	14.2	47.9
24	55.5	32.4	12.1	44.5
48	53.2	23.1	12.4	35.5

Table B29b. VFA concentrations (%) in caecal liquor following intravenous administration of ampicillin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	37.9	12.6	1.1	9.3	3.4	0.0	64.3
0.25	0.0	33.8	9.4	1.1	6.8	0.0	0.0	51.1
0.5	0.0	36.0	9.0	0.0	6.6	0.0	0.0	51.6
0.75	0.0	39.9	11.0	1.3	7.9	0.0	3.1	63.2
1	0.0	34.8	8.0	1.6	7.7	0.0	0.0	52.1
1.5	0.0	32.8	9.1	0.0	5.5	0.0	2.0	49.4
2	0.0	42.9	11.5	1.3	9.0	0.0	1.7	66.4
4	0.0	45.0	11.5	0.8	8.0	0.0	0.0	65.3
6	0.0	33.1	7.9	0.0	6.0	0.0	0.0	47.0
8	0.0	37.2	10.2	0.0	7.4	0.0	0.0	54.8
12	0.0	36.7	9.0	0.7	7.7	0.0	0.0	54.1
24	2.1	40.9	17.8	0.0	7.5	0.0	0.0	66.2
48	0.6	31.9	10.1	0.7	6.0	0.0	0.0	48.7

Table B30a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of ampicillin to pony II2

Time (h)	A	P	B	P+B
0	58.9	19.6	14.5	34.1
0.25	66.1	18.4	13.3	31.7
0.5	69.8	17.4	12.8	30.2
0.75	63.1	17.4	12.5	29.9
1	66.8	15.4	14.8	30.1
1.5	66.4	18.4	11.1	29.6
2	64.6	17.3	13.6	30.9
4	68.9	17.6	12.3	29.9
6	70.4	16.8	12.8	29.6
8	67.9	18.6	13.5	32.1
12	67.8	16.6	14.2	30.9
24	61.8	26.9	11.3	38.2
48	65.5	20.7	12.3	33.1

Table B30b. VFA concentrations (%) in caecal liquor following intravenous administration of ampicillin to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	7.5	0.0	0.0	15.8	0.0	0.0	23.3
24	0.0	15.4	7.6	0.0	35.2	0.0	5.4	63.6
48	0.0	6.1	5.3	0.0	13.2	0.0	0.0	24.6

Table B31. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	7.5	0.0	19.6	0.0	0.0	27.1
24	0.0	4.5	0.0	0.0	47.0	6.1	0.0	57.6
48	0.0	0.0	0.0	0.0	21.9	0.0	0.0	21.9

Table B32. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	6.5	4.6	0.0	21.4	0.0	0.0	32.5
24	0.0	30.3	7.5	0.0	57.5	0.0	0.0	95.3
48	0.0	10.8	4.9	0.0	23.2	0.0	2.9	41.8

Table B33. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	8.3
24	0.0	0.0	0.0	5.8	0.0	0.0	0.0	5.8
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table B34. SCFA concentrations (mmol/kg) in faeces following intravenous administration of ampicillin to pony II2

Time (h)	I1	I2	II1	II2
0	19.33	33.16	20.84	18.46
24	22.70	33.83	22.17	17.73
48	20.94	18.19	22.12	18.85

Table B35. Faecal dry matter content (%) following intravenous administration of ampicillin to ponies

	I1			I2		
Time (h)	0	24	48	0	24	48
urea (mmol/l)	1.4	3.3	2.7	4.3	5.7	2.4
sodium (mmol/l)	143	138	141	135	135	135
potassium (mmol/l)	4.3	3.9	3.6	3.9	3.8	4.2
chloride (mmol/l)	101	99	101	95	94	100
calcium (mmol/l)	2.66	2.99	3.05	2.98	2.92	3.05
magnesium (mmol/l)	0.62	0.60	0.62	0.56	0.60	0.70
phosphate (mmol/l)	0.95	0.61	0.88	0.44	0.45	1.32
creatinine (µmol/l)	142	157	159	142	148	154
bilirubin (µmol/l)	6	11	8	21	20	7
SAP (U/l)	381	380	366	500	466	465
AST (U/l)	269	276	264	291	289	278
GGT (U/l)	13	8	14	21	23	17
total protein (g/l)	65	69	67	68	68	69
albumin (g/l)	27	28	28	27	27	29
globulin (g/l)	38	41	39	41	41	40

Table B36. Plasma biochemistry following intravenous administration of ampicillin to pony I

	II1			II2		
Time (h)	0	24	48	0	24	48
urea (mmol/l)	0.4	0.9	0.9	3.1	2.8	1.8
sodium (mmol/l)	141	140	141	138	137	137
potassium (mmol/l)	4.1	3.7	3.3	3.2	3.6	3.8
chloride (mmol/l)	104	105	103	97	95	101
calcium (mmol/l)	3.16	3.18	2.98	2.85	2.84	2.98
magnesium (mmol/l)	0.62	0.71	0.68	0.59	0.60	0.71
phosphate (mmol/l)	1.04	0.60	0.92	0.54	0.41	0.61
creatinine (µmol/l)	117	133	134	108	113	105
bilirubin (µmol/l)	6	12	11	16	19	13
SAP (U/l)	450	461	459	631	585	526
AST (U/l)	348	363	375	414	414	498
GGT (U/l)	42	44	45	55	53	58
total protein (g/l)	72	73	72	69	66	71
albumin (g/l)	32	32	32	29	30	32
globulin (g/l)	40	41	40	31	36	39

Table B37. Plasma biochemistry following intravenous administration of ampicillin to pony II

	I1			I2		
Time (h)	0	24	48	0	24	48
WCC (x10^9/l)	8.3	6.7	7.5	6.0	5.5	8.6
RCC (x10^{12}/l)	6.56	5.77	5.98	5.83	5.86	6.13
Hb (g/dl)	11.4	9.9	10.4	10.4	10.2	11.0
Hct (l/l)	0.333	0.291	0.303	0.294	0.297	0.306
MCV (fl)	51.0	50.0	51.0	50.0	51.0	50.0
MCH (pg)	17.3	17.1	17.3	17.6	17.4	17.9
MCHC (g/dl)	34.2	34.0	34.3	35.3	34.3	35.9
PLTS (10^9/l)	-	107	106	97	115	122
MPV (fl)	-	6.4	6.1	6.0	6.0	6.2
PCT (%)	-	0.068	0.064	0.058	0.069	0.075
PDW	-	17.1	16.3	18.3	16.6	17.7
Neu (%)	44.8	45.0	41.0	65.0	50.4	35.0
Lym (%)	54.4	52.9	55.4	31.0	46.5	58.9
Mon (%)	0.5	0.0	1.5	4.0	2.0	3.0
Eos (%)	0.4	1.0	1.1	0.0	0.5	2.6
Bas (%)	0.0	1.0	1.1	0.0	0.5	0.5

Table B38. Haematology parameters following intravenous administration of ampicillin to pony I

	II1			II2		
Time (h)	0	24	48	0	24	48
WCC (x10^9/l)	7.5	6.9	6.1	5.0	5.0	4.6
RCC (x10^{12}/l)	7.46	7.06	6.72	6.56	6.84	7.06
Hb (g/dl)	11.7	11.2	10.4	10.4	10.3	11.2
Hct (l/l)	0.342	0.318	0.303	0.288	0.303	0.312
MCV (fl)	46.0	45.0	45.0	44.0	44.0	44.0
MCH (pg)	15.6	15.8	15.4	15.6	15.0	15.8
MCHC (g/dl)	34.2	35.2	34.3	36.1	33.9	35.8
PLTS (10^9/l)	83	105	106	102	123	109
MPV (fl)	7.1	6.8	6.5	6.5	6.8	6.8
PCT (%)	0.058	0.071	0.068	0.066	0.083	0.074
PDW	18.3	16.1	16.9	16.9	16.1	16.1
Neu (%)	46.9	43.0	51.0	67.0	62.0	52.6
Lym (%)	50.9	51.9	47.0	31.0	33.0	42.0
Mon (%)	0.5	1.4	0.0	1.0	3.0	5.0
Eos (%)	1.1	3.0	1.5	0.0	1.0	0.0
Bas (%)	0.5	0.6	0.5	1.0	1.0	0.0

Table B39. Haematology parameters following intravenous administration of ampicillin to pony II

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	1.67	0.38	0.00	0.24
0.5	2.30	1.16	0.67	0.74
0.75	2.05	1.10	1.96	0.59
1	1.55	1.02	1.81	0.42
1.5	0.64	0.47	0.69	0.16
2	0.30	0.33	0.23	0.00
4	0.00	0.00	0.00	0.00

Table B40. Plasma concentrations (µg/ml) of ampicillin following oral administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	0.00
0.75	0.76	0.00	0.00	0.00
1	45.24	0.00	0.00	0.00
1.5	100.67	15.03	0.00	0.00
2	101.31	144.53	0.00	0.00
4	65.07	0.00	19.11	0.00
6	27.95	0.00	47.40	3.63
8	23.55	0.00	46.93	0.37
12	0.00	0.00	0.00	0.00

Table B41. Caecal liquor concentrations (µg/ml) of ampicillin following oral administration to ponies

Time (h)	I1	I2	II1	II2
coliforms				
0	1.60E+11	1.40E+05	4.00E+05	4.00E+05
24	1.10E+11	1.00E+08	4.40E+11	2.20E+10
48	1.20E+11	2.00E+06	6.10E+11	1.00E+09
72	2.00E+07	1.00E+06	3.00E+06	1.70E+06
96	2.00E+05	1.00E+07	2.00E+05	5.00E+04
168	1.40E+05	1.10E+06	2.00E+04	2.30E+04
streptococci				
0	2.00E+10	1.00E+06	1.00E+07	1.00E+07
24	1.40E+10	1.00E+07	1.00E+10	2.00E+07
48	6.00E+10	1.00E+07	4.00E+10	1.00E+09
72	3.00E+08	5.00E+05	1.30E+07	4.00E+08
96	3.00E+07	2.00E+05	1.00E+07	3.00E+05
168	1.00E+06	8.00E+04	2.00E+05	7.00E+03
lactobacilli				
0	2.00E+10	1.00E+06	1.00E+09	1.00E+09
24	1.00E+10	1.00E+09	5.00E+07	1.00E+07
48	1.10E+11	1.00E+06	1.00E+09	1.00E+10
72	2.00E+06	3.00E+05	6.00E+07	6.00E+06
96	2.00E+07	1.00E+06	4.00E+06	8.00E+08
168	1.00E+06	5.00E+04	2.00E+05	1.00E+05
Bacteroides spp.				
0	1.70E+11	1.30E+07	5.00E+08	5.00E+08
24	2.10E+11	4.00E+08	5.80E+11	1.10E+07
48	1.00E+12	1.60E+08	1.00E+12	2.00E+10
72	1.90E+08	1.10E+07	3.40E+08	2.00E+10
96	1.10E+10	5.90E+07	3.30E+10	2.00E+10
168	1.30E+07	3.50E+07	3.20E+07	3.00E+10
Clostridium spp.				
0	6.00E+05	-	-	-
24	8.00E+07	1.20E+07	3.00E+05	3.00E+04
48	3.00E+10	1.00E+05	2.00E+06	3.00E+05
72	-	1.00E+04	-	1.30E+06
96	1.00E+06	1.00E+05	1.00E+06	1.00E+03
168	-	3.00E+04	1.00E+03	2.00E+03

Table B42. Counts of viable bacteria per ml caecal liquor following oral administration of ampicillin to ponies

Time (h)	I1	I2	II1	II2
0	7.0	6.7	7.0	6.7
0.25	6.9	6.9	6.8	6.7
0.5	6.8	6.9	6.8	6.6
0.75	6.8	6.8	6.9	6.7
1	6.9	6.9	6.9	6.6
1.5	6.7	6.8	6.9	6.9
2	6.9	6.9	6.9	6.8
4	7.0	7.5	6.8	6.9
6	7.3	7.8	6.9	7.5
8	7.3	7.6	6.9	7.0
12	7.5	5.7	7.0	6.6
24	6.7	7.3	6.8	7.3
28	7.0	6.7	6.9	7.0
32	6.8	6.7	7.1	7.4
48	7.0	6.8	7.1	7.2
72	6.4	6.5	7.3	7.0
96	7.0	8.1	7.1	7.5
168	6.7	7.9	6.7	7.4

Table B43. Caecal liquor pH following oral administration of ampicillin to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	13.6	31.1	28.3	0.0	19.9	0.0	0.0	79.3
0.25	6.5	18.8	16.7	0.0	5.8	0.0	0.0	41.3
0.5	6.2	37.8	30.0	0.0	21.2	0.0	0.0	89.0
0.75	4.3	28.7	21.2	0.0	20.1	0.0	0.0	70.0
1	2.4	24.0	12.3	0.0	17.8	0.0	0.0	54.1
1.5	2.7	32.6	16.5	0.0	18.5	0.0	0.0	67.6
2	4.0	27.9	14.1	0.0	29.4	0.0	0.0	71.4
4	9.8	12.3	10.8	0.0	43.2	0.0	0.0	66.3
6	23.5	6.9	24.6	0.0	28.2	0.0	0.0	59.7
8	35.4	34.7	53.1	0.0	13.6	0.0	0.0	101.4
12	40.3	4.4	99.9	0.0	11.6	2.9	0.0	118.8
24	42.7	7.1	0.0	0.0	28.1	0.0	0.0	35.2
28	41.8	12.2	7.5	0.0	24.5	0.0	0.0	44.2
32	26.6	17.1	22.6	0.0	28.2	1.4	0.0	69.3
48	0.0	21.6	25.8	0.0	46.1	0.0	0.0	93.5
72	3.3	22.7	41.5	0.0	33.1	2.5	1.9	101.7
96	0.0	12.3	17.3	0.0	13.6	0.0	0.0	43.2
168	0.7	19.3	10.1	0.0	7.7	0.0	0.0	37.1

Table B44a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of ampicillin to pony I1

Time (h)	A	P	B	P+B
0	39.2	35.7	25.1	60.8
0.25	45.5	40.4	14.0	54.5
0.5	42.5	33.7	23.8	57.5
0.75	41.0	30.3	28.7	59.0
1	44.4	22.7	32.9	55.6
1.5	48.2	24.4	27.4	51.8
2	39.1	19.7	41.2	60.9
4	18.6	16.3	65.2	81.4
6	11.6	41.2	47.2	88.4
8	34.2	52.4	13.4	65.8
12	3.7	84.1	9.8	93.9
24	20.2	0.0	79.8	79.8
28	27.6	17.0	55.4	72.4
32	24.3	32.6	40.7	73.3
48	23.1	27.6	49.3	76.9
72	22.3	40.8	32.5	73.4
96	28.5	40.0	31.5	71.5
168	52.0	27.2	20.8	48.0

Table B44b. VFA concentrations (%) in caecal liquor following oral administration of ampicillin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.7	19.3	10.1	0.0	7.7	0.0	0.0	37.1
0.25	0.5	19.0	8.7	0.0	29.4	0.0	0.0	57.1
0.5	0.4	20.5	9.6	0.0	21.9	0.0	0.0	52.0
0.75	1.9	20.5	9.4	0.0	19.0	0.0	0.0	48.9
1	3.1	22.8	10.5	0.0	19.8	0.8	0.9	54.8
1.5	2.2	22.3	11.4	0.0	14.2	0.0	0.0	47.9
2	3.6	23.8	12.7	0.0	27.1	0.0	0.0	63.6
4	9.1	18.8	17.4	0.0	26.6	0.0	0.0	62.8
6	24.5	13.8	19.9	0.0	17.2	0.0	0.0	50.9
8	51.6	9.0	15.9	0.0	1.2	0.0	0.0	26.1
12	14.0	10.6	0.0	1.8	24.4	0.0	0.0	36.8
24	4.7	17.7	1.7	0.0	28.6	0.0	0.0	48.0
28	4.5	25.3	5.9	0.0	20.1	0.4	0.0	51.7
32	6.5	26.2	8.2	0.0	23.2	0.0	0.0	57.6
48	7.3	30.2	16.7	0.0	30.8	0.0	0.0	77.7
72	0.9	21.5	10.7	0.0	9.4	0.0	1.9	43.5
96	0.4	23.3	12.5	0.0	31.2	0.0	0.0	67.0
168	0.2	18.0	7.4	0.0	16.3	0.0	0.0	41.7

Table B45a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of ampicillin to pony I2

Time (h)	A	P	B	P+B
0	52.0	27.2	20.8	48.0
0.25	33.3	15.2	51.5	66.7
0.5	39.4	18.5	42.1	60.6
0.75	41.9	19.2	38.9	58.1
1	41.6	19.2	36.1	55.3
1.5	46.6	23.8	29.6	53.4
2	37.4	20.0	42.6	62.6
4	29.9	27.7	42.4	70.1
6	27.1	39.1	33.8	72.9
8	34.5	60.9	4.6	65.5
12	28.8	0.0	66.3	66.3
24	36.9	3.5	59.6	63.1
28	48.9	11.4	38.9	50.3
32	45.5	14.2	40.3	54.5
48	38.9	21.5	39.6	61.1
72	49.4	24.6	21.6	46.2
96	34.8	18.7	46.6	65.2
168	43.2	17.7	39.1	56.8

Table B45b. VFA concentrations (%) in caecal liquor following oral administration of ampicillin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.5	33.7	7.3	0.0	12.5	1.4	0.0	54.9
0.25	0.6	30.1	6.3	0.5	8.9	0.9	0.0	46.7
0.5	0.0	27.1	5.8	0.6	5.5	1.1	0.0	40.1
0.75	0.3	30.2	5.6	0.0	6.0	0.0	0.0	41.8
1	0.5	29.3	6.4	0.2	6.2	1.7	0.0	43.8
1.5	0.0	34.9	7.2	0.0	7.7	0.6	0.0	50.4
2	0.0	45.2	10.2	0.3	11.7	0.0	0.0	67.4
4	0.9	35.9	6.4	0.6	11.7	0.0	0.0	54.6
6	3.1	32.0	3.5	0.0	18.0	0.0	0.4	53.9
8	5.7	28.8	1.9	0.0	25.5	0.0	0.0	56.2
12	14.8	17.5	0.0	0.0	20.1	0.0	0.0	37.6
24	36.4	25.3	0.0	0.0	37.0	0.0	0.0	62.3
28	34.3	14.5	1.0	0.0	20.7	0.0	0.0	36.2
32	39.6	14.0	2.9	0.0	18.4	0.0	0.0	35.3
48	0.7	22.9	10.0	0.3	2.7	3.0	0.0	38.9
72	0.7	39.1	11.3	0.2	22.3	1.4	0.0	74.3
96	0.9	42.3	14.0	0.5	18.3	0.6	0.0	75.7
168	0.7	28.1	9.4	0.3	13.4	0.3	0.0	51.2

Table B46a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of ampicillin to pony II1

Time (h)	A	P	B	P+B
0	61.4	13.3	22.8	36.1
0.25	64.5	13.5	19.1	32.5
0.5	67.6	14.5	13.7	28.2
0.75	72.2	13.4	14.4	27.8
1	66.9	14.6	14.2	28.8
1.5	69.2	14.3	15.3	29.6
2	67.1	15.1	17.4	32.5
4	65.8	11.7	21.4	33.2
6	59.4	6.5	33.4	39.9
8	51.2	3.4	45.4	48.8
12	46.5	0.0	53.5	53.5
24	40.6	0.0	59.4	59.4
28	40.1	2.8	57.2	59.9
32	39.7	8.2	52.1	60.3
48	58.9	25.7	6.9	32.6
72	52.6	15.2	30.0	45.2
96	55.9	18.5	24.2	42.7
168	54.9	18.3	25.5	43.7

Table B46b. VFA concentrations (%) in caecal liquor following oral administration of ampicillin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.7	28.1	9.4	0.3	13.4	0.0	0.0	51.2
0.25	0.2	29.8	10.9	0.3	15.2	2.1	1.4	59.7
0.5	0.2	27.9	10.6	0.1	9.9	0.0	3.1	51.6
0.75	0.4	36.2	12.4	0.0	11.7	0.9	0.0	61.2
1	0.4	27.8	9.9	0.0	6.6	1.5	0.0	45.8
1.5	0.9	32.3	11.8	0.0	10.9	1.2	0.0	56.2
2	0.5	37.1	12.7	0.0	11.2	1.3	0.0	62.3
4	0.4	38.4	14.0	0.0	15.4	1.5	0.0	69.3
6	1.0	26.7	7.2	0.0	18.5	1.2	0.0	53.6
8	6.3	29.9	3.8	0.0	24.2	1.3	0.0	59.2
12	40.5	27.9	0.3	0.0	21.9	0.0	0.0	50.1
24	35.3	20.4	0.7	0.0	22.5	0.0	0.0	43.6
28	15.3	22.0	1.9	0.0	24.9	1.2	0.0	50.0
32	13.6	24.3	2.6	0.0	16.2	1.7	0.0	44.8
48	10.2	24.0	9.0	0.0	18.4	2.8	0.0	54.2
72	1.1	36.3	13.3	0.0	57.7	0.0	0.0	107.3
96	0.2	32.8	12.0	0.7	24.9	1.6	0.0	72.0
168	0.5	29.0	9.7	0.4	13.3	0.0	0.0	52.4

Table B47a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of ampicillin to pony II2

Time (h)	A	P	B	P+B
0	54.9	18.3	25.5	43.7
0.25	54.1	20.5	19.2	39.7
0.5	54.1	20.5	19.2	39.7
0.75	59.2	20.3	19.1	39.4
1	60.7	21.6	14.4	36.0
1.5	57.5	21.0	19.4	40.4
2	59.6	20.4	18.0	38.4
4	55.4	20.2	22.2	42.4
6	49.8	13.4	34.5	47.9
8	50.5	6.4	40.9	47.3
12	55.7	0.6	43.7	44.3
24	46.8	1.6	51.6	53.2
28	44.0	3.8	49.8	53.6
32	54.2	5.8	36.2	42.0
48	44.3	16.6	33.9	50.6
72	33.8	12.4	53.8	66.2
96	45.6	16.7	34.6	51.3
168	55.3	18.5	25.4	43.9

Table B47b. VFA concentrations (%) in caecal liquor following oral administration of ampicillin to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.9	14.8	5.1	0.0	12.5	0.0	0.0	32.4
24	50.2	36.2	0.0	0.0	15.7	0.0	0.0	51.9
48	16.4	17.9	0.0	0.0	27.6	0.0	0.0	45.5
72	1.9	0.0	0.0	0.0	57.4	0.0	0.0	57.4
96	1.6	2.9	4.7	0.0	11.6	2.5	0.0	21.7
168	0.0	33.4	12.2	0.0	65.7	0.0	0.0	111.3

Table B48. SCFA concentrations (mmol/kg) in faeces following oral administration of ampicillin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	33.4	12.2	0.0	65.7	0.0	0.0	111.3
24	4.5	31.1	5.9	0.0	34.9	2.5	0.0	74.4
48	4.5	21.5	4.7	0.0	22.0	0.0	0.0	48.2
72	3.5	1.7	0.0	0.0	0.0	0.0	0.0	1.7
96	3.7	22.1	0.0	0.0	33.2	0.0	0.0	55.3
168	2.3	23.5	2.7	0.0	31.5	0.0	0.0	57.7

Table B49. SCFA concentrations (mmol/kg) in faeces following oral administration of ampicillin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	7.9	0.0	0.0	8.2	0.0	0.0	16.1
24	64.2	24.2	0.0	0.0	31.2	0.0	0.0	55.4
48	37.4	18.1	0.0	0.0	27.7	0.0	0.0	45.8
72	8.0	0.0	0.0	0.0	25.5	0.0	0.0	25.5
96	5.9	0.0	0.0	0.0	40.5	2.8	0.0	43.3
168	3.7	8.1	0.0	0.0	31.5	0.0	0.0	39.6

Table B50. SCFA concentrations (mmol/kg) in faeces following oral administration of ampicillin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.7	8.1	0.0	0.0	31.5	0.0	0.0	39.6
24	35.6	10.7	0.0	0.0	106.9	0.0	0.0	117.6
48	43.6	12.4	0.0	0.0	23.8	0.0	0.0	36.2
72	4.5	3.2	0.0	0.0	187.2	0.0	0.0	190.4
96	5.2	15.4	5.2	0.0	79.6	0.0	0.0	100.2
168	3.9	29.1	0.0	0.0	66.5	0.0	0.0	95.6

Table B51. SCFA concentrations (mmol/kg) in faeces following oral administration of ampicillin to pony II2

Time (h)	I1	I2	II1	II2
0	19.15	21.82	20.62	20.18
24	20.56	27.58	18.26	17.49
48	21.67	21.96	16.87	18.94
72	22.59	22.66	21.05	20.76
96	23.43	21.96	20.81	23.43
168	21.82	22.82	20.18	22.07

Table B52. Faecal dry matter content (%) following oral administration of ampicillin to ponies

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.16	0.04±0.04
1	0.43	0.49	0.28	1.24	0.61±0.21
5	5.68	5.32	4.36	6.32	5.42±0.41
10	11.50	9.65	9.25	11.98	10.60±0.67
20	23.61	21.40	19.56	24.52	22.27±1.12
40	44.89	38.11	33.69	47.51	41.05±3.15
80	66.06	61.29	59.32	73.61	65.07±3.18

Table B53a. Concentrations (µg/ml) of ampicillin in caecal liquor following incubation *in vitro* for 3 h

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.00	0.00±0.00
1	0.00	0.00	0.00	0.00	0.00±0.00
5	0.58	4.93	0.65	0.00	1.54±1.14
10	3.93	0.00	2.93	0.00	1.72±1.01
20	11.62	0.00	8.45	3.24	5.83±2.60
40	17.73	38.29	16.73	12.38	21.28±5.79
80	41.92	74.18	35.29	13.09	41.12±12.63

Table B53b. Concentrations (µg/ml) of ampicillin in caecal liquor following incubation *in vitro* for 24 h

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.8	21.5	10.5	0.0	10.1	1.9	0.0	44.0
0.25	2.4	22.3	8.6	2.3	7.3	2.7	2.3	45.5
1	1.0	26.8	10.1	0.6	8.4	0.0	0.0	45.9
5	2.8	31.8	15.1	2.4	11.1	2.4	4.6	67.4
10	1.5	26.5	14.4	0.0	7.8	0.0	0.0	48.7
20	1.3	25.4	10.2	1.0	7.7	1.2	0.0	45.5
40	0.5	28.8	10.1	0.0	7.8	0.0	0.0	46.7
80	2.0	24.8	10.5	1.3	11.0	1.8	2.3	51.7

Table B54a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 3 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.8	23.4	9.1	0.7	5.3	0.0	0.0	38.5
0.25	1.0	24.5	9.5	1.1	5.3	1.3	1.7	43.4
1	0.0	29.3	11.3	0.0	6.9	0.0	0.0	47.5
5	2.3	31.9	12.4	2.7	8.6	3.3	3.9	62.8
10	0.3	33.6	12.7	0.0	7.5	0.0	0.0	53.8
20	1.9	26.9	11.1	1.6	5.8	2.3	0.0	47.7
40	0.4	24.1	8.6	0.0	4.6	0.0	0.0	37.3
80	1.5	26.3	9.3	1.5	5.8	1.8	0.0	44.7

Table B54b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 24 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.6	33.1	11.8	0.4	7.7	1.3	0.0	54.3
0.25	2.4	34.2	15.3	1.8	9.8	2.5	0.0	63.6
1	1.1	33.9	13.3	0.0	11.3	1.8	0.0	60.3
5	1.5	35.2	14.2	1.4	9.8	2.3	0.0	62.9
10	0.8	39.5	16.1	0.4	15.7	2.8	2.2	76.7
20	1.6	38.8	15.3	1.3	12.8	2.2	0.0	70.4
40	0.4	52.0	13.5	0.0	16.9	0.0	0.0	82.4
80	1.2	37.8	16.3	1.2	12.5	1.7	2.0	71.5

Table B55a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 3 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	36.4	14.3	0.0	7.7	2.2	0.0	60.6
0.25	1.5	36.8	13.9	1.6	7.7	3.4	2.6	66.0
1	0.0	35.5	13.5	0.0	5.8	3.7	0.0	58.5
5	2.1	36.7	19.5	2.4	8.4	3.5	3.4	73.9
10	0.4	40.9	16.3	0.5	9.3	2.7	2.0	71.7
20	1.2	52.8	19.4	1.8	14.1	3.3	3.4	94.8
40	0.0	39.9	15.1	0.8	8.6	0.0	0.0	64.4
80	1.0	38.2	14.2	1.3	9.2	2.8	2.2	67.9

Table B55b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 24 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.5	25.5	10.7	0.0	4.6	0.0	0.0	40.8
0.25	1.3	27.8	11.1	0.8	8.1	0.8	0.0	48.6
1	1.5	27.3	11.9	1.5	8.8	2.1	0.0	51.6
5	0.0	28.2	10.8	0.0	7.4	1.5	0.0	47.9
10	2.4	28.5	11.9	2.1	8.7	2.9	4.0	58.1
20	0.6	28.8	9.9	0.0	9.3	0.0	0.0	48.0
40	2.5	31.6	13.6	2.8	10.5	1.9	3.4	63.8
80	0.5	33.2	14.5	0.0	13.2	0.0	0.0	60.9

Table B56a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 3 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	2.3	33.4	13.7	2.0	0.7	2.9	2.9	55.6
0.25	0.1	29.0	10.5	0.0	4.3	2.5	0.0	46.3
1	1.3	26.9	10.8	1.5	6.4	2.3	3.6	51.5
5	0.3	30.8	10.5	0.4	3.7	0.0	0.0	45.4
10	2.2	36.6	12.4	2.2	7.9	4.2	4.0	67.3
20	0.6	31.7	12.0	1.2	5.6	2.3	0.0	52.8
40	2.4	34.4	14.3	2.6	8.9	3.9	0.0	64.1
80	0.0	28.7	10.5	0.7	4.8	1.2	0.0	45.9

Table B56b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 24 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	37.2	11.6	0.0	6.7	0.0	0.0	55.5
0.25	0.0	34.4	11.5	0.0	9.3	0.0	0.0	55.2
1	0.0	38.7	13.7	0.0	11.4	1.4	0.0	65.2
5	0.0	27.9	9.8	0.0	9.7	0.3	0.0	47.7
10	0.9	29.8	9.6	0.3	10.5	0.0	0.0	50.2
20	0.0	30.9	8.9	0.0	11.6	0.2	0.0	51.6
40	0.4	36.1	10.9	1.3	10.8	0.5	0.0	59.6
80	0.0	27.8	8.8	0.0	7.0	0.5	0.0	44.1

Table B57a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 3 h (replicate 4)

Conc. (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	30.9	9.2	0.7	4.1	0.0	0.0	44.9
0.25	0.2	35.7	11.8	0.0	10.7	0.3	0.0	58.5
1	0.0	32.8	12.5	0.0	10.6	0.3	0.0	56.2
5	0.0	34.1	13.4	0.0	10.3	0.7	0.0	58.5
10	0.0	31.7	12.5	0.5	10.0	0.7	0.0	55.4
20	0.1	38.2	12.3	0.0	10.6	2.2	0.0	63.3
40	0.5	36.6	13.0	0.0	11.4	0.0	0.0	61.0
80	0.5	40.7	17.5	0.0	12.8	0.3	0.0	71.3

Table B57b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with ampicillin for 24 h (replicate 4)

	3 h		24 h	
Conc (µg/ml)	L	Total	L	Total
0	0.5±0.2	48.7±3.7	0.8±0.5	49.9±5.0
0.25	1.5±0.6	53.2±4.0	0.7±0.3	53.6±5.3
1	0.9±0.3	55.8±4.3	0.3±0.3	53.4±2.5
5	1.1±0.7	56.5±5.1	1.2±0.6	60.2±5.9
10	1.4±0.4	58.4±6.4	0.7±0.5	62.1±4.4
20	0.9±0.4	53.9±5.7	1.0±0.4	64.7±10.6
40	1.0±0.5	63.1±7.4	0.8±0.5	56.7+6.5
80	0.9±0.4	57.1±5.9	0.8±0.3	57.5±7.1

Table B58. SCFA concentrations (mmol/l) (mean±SEM) in caecal liquor following *in vitro* incubation with ampicillin

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
1	1.05	0.99	0.92	0.85	0.95±0.04
2	2.11	1.98	2.65	2.19	2.23±0.15
5	5.56	4.87	4.60	4.77	4.95±0.21
10	9.49	9.76	8.42	8.24	8.98±0.38

Table B59. Concentrations (µg/ml) of ampicillin following incubation *in vitro* at pH 1.9 for 1 h

Conc. (µg/ml)	1	2	3	4	5	6	mean±SEM
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
10	6.88	8.13	8.48	8.04	6.92	0.30	6.46±1.26
25	30.77	24.38	22.98	26.14	23.19	24.73	25.37±1.18
50	62.96	52.74	62.26	46.83	44.47	80.62	58.31±5.44
100	127.98	105.68	86.96	89.87	87.82	141.39	106.62±9.46

Table B60a. Concentrations (µg/ml) of ampicillin following incubation *in vitro* with hay at pH 1.9 for 3 h

Conc. (µg/ml)	1	2	3	4	5	6	mean±SEM
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
10	0.21	3.85	1.57	1.73	2.41	4.72	2.42±0.67
25	7.55	5.99	8.23	11.17	10.28	10.78	9.00±0.84
50	10.98	15.53	15.78	27.98	29.94	32.57	22.13±3.71
100	51.67	32.69	43.68	59.56	89.94	74.92	58.74±8.55

Table B60b. Concentrations (µg/ml) of ampicillin following incubation *in vitro* with hay at pH 7.0 for 3 h

Appendix C - Amikacin

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	67.79	30.20	78.48	58.82±14.64
0.083	49.64	21.23	60.16	43.68±11.63
0.25	36.88	17.90	55.80	36.86±10.94
0.5	27.53	17.22	41.58	28.78±7.06
0.75	27.33	15.70	33.16	25.40±5.13
1.0	26.60	14.28	29.56	23.48±4.68
1.5	21.46	12.97	26.10	20.18±3.84
2	19.60	12.83	16.38	16.27±1.96
4	13.49	11.33	8.56	11.13±1.43
6	8.71	12.16	3.46	8.11±2.53
8	6.66	7.70	1.76	5.37±1.83
12	2.39	2.92	0.47	1.93±0.74
24	0.00	0.00	0.00	0.00±0.00

Table C1. Plasma concentrations (μg/ml) of amikacin following intravenous administration to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.033	56.59	64.00	65.64	62.47	62.17±2.26
0.083	47.14	53.95	51.11	56.50	52.17±2.79
0.25	38.95	41.25	38.54	40.51	39.81±0.68
0.5	27.13	39.44	22.23	28.96	29.44±3.83
0.75	25.49	33.99	19.35	23.80	25.66±3.15
1	23.15	28.33	14.33	17.86	20.92±3.02
1.5	15.45	18.00	9.21	12.14	13.70±1.70
2	12.70	14.90	7.14	9.83	11.14±1.47
4	4.20	7.79	2.51	3.42	4.48±1.35
6	2.01	4.73	0.99	1.11	2.21±1.09
8	0.88	2.67	0.57	0.54	1.16±0.66
24	0.00	0.00	0.00	0.00	0.00±0.00

Table C2. Plasma concentrations (μg/ml) of amikacin following intravenous administration to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.033	71.83	54.79	85.07	70.56±8.77
0.083	57.80	38.41	76.65	57.62±11.04
0.25	45.90	29.72	45.01	40.21±5.25
0.5	40.90	23.78	39.78	34.82±5.53
0.75	28.06	21.38	32.05	27.16±3.11
1	26.56	19.53	31.34	25.81±3.43
1.5	23.66	14.56	27.89	22.04±3.93
2	17.23	9.79	24.62	17.21±4.28
4	10.32	5.75	11.49	9.19±1.75
6	5.75	1.91	7.67	5.11±1.70
8	3.95	0.82	4.24	3.00±1.10
12	0.82	0.10	1.19	0.71±0.32
24	0.00	0.00	0.00	0.00±0.00

Table C3. Plasma concentrations (μg/ml) of amikacin following intravenous administration to donkeys

Parameter	Horse 3	Horse 4	Horse 5
t1/2 B2 (h)	0.05	0.21	0.22
t1/2 B1 (h)	3.24	5.01	1.82
Cp0 (μg/ml)	88.66	28.77	77.34
Vc (ml/kg)	67.67	208.53	77.58
AUCobs (μg.h/ml)	159.80	129.59	119.07
AUMCobs (μg.h^2/ml)	687.96	689.51	283.99
AUC (μg.h/ml)	155.65	132.03	116.38
AUMC (μg.h^2/ml)	709.14	932.14	278.20
MRT (h)	4.31	5.32	2.39
Vdarea (ml/kg)	180.25	328.68	135.67
Vdss (ml/kg)	175.63	320.86	123.24
CLb (ml/h.kg)	38.55	45.45	51.56
kel (/h)	0.57	0.22	0.67
k21 (/h)	5.20	2.13	1.78
k12 (/h)	8.30	1.15	1.05

Table C4. Disposition kinetics of amikacin following intravenous administration to horses

Parameter	Pony 7	Pony 8	Pony 9	Pony 10
t1/2 B2 (h)	0.17	0.42	0.16	0.19
t1/2 B1 (h)	1.48	2.59	1.44	1.34
Cp0 (μg/ml)	57.57	63.65	70.92	66.95
Vc (ml/kg)	104.23	94.27	84.60	89.62
AUCobs (μg.h/ml)	82.31	123.84	58.90	70.44
AUMCobs (μg.h^2/ml)	183.83	386.76	112.13	130.76
AUC (μg.h/ml)	77.51	110.50	55.58	66.83
AUMC (μg.h^2/ml)	154.30	335.89	93.80	111.36
MRT (h)	2.23	3.12	1.90	1.86
Vdarea (ml/kg)	165.49	202.84	223.80	173.00
Vdss (ml/kg)	154.08	165.05	182.18	149.61
CLb (ml/h.kg)	77.41	54.30	108.00	89.78
kel (/h)	0.74	0.58	1.28	1.00
k21 (/h)	2.51	0.77	1.61	1.85
k12 (/h)	1.20	0.58	1.86	1.24

Table C5. Disposition kinetics of amikacin following intravenous administration to ponies

Parameter	Donkey 14	Donkey 15	Donkey 16
t1/2 B2 (h)	0.14	0.03	0.07
t1/2 B1 (h)	2.23	1.48	2.34
Cp0 (μg/ml)	74.18	79.03	110.69
Vc (ml/kg)	80.89	75.92	54.21
AUCobs (μg.h/ml)	131.17	70.66	158.05
AUMCobs (μg.h^2/ml)	409.29	149.91	508.38
AUC (μg.h/ml)	129.15	68.33	151.31
AUMC (μg.h^2/ml)	392.76	141.03	488.82
MRT (h)	3.12	2.12	3.22
Vdarea (ml/kg)	149.16	187.50	133.90
Vdss (ml/kg)	141.28	181.24	128.11
CLb (ml/h.kg)	46.46	87.81	39.66
kel (/h)	0.57	1.16	0.73
k21 (/h)	2.70	8.36	4.08
k12 (/h)	2.02	11.59	5.57

Table C6. Disposition kinetics of amikacin following intravenous administration to donkeys

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
coliforms				
0	2.00E+07	1.00E+05	1.00E+05	6.73E+06±6.63E+06
24	1.00E+04	4.00E+03	1.00E+06	3.38E+05±3.31E+05
48	1.00E+05	1.00E+05	1.00E+05	1.00E+05±0.00E+05
streptococci				
0	1.00E+10	5.00E+06	3.00E+07	3.35E+09±3.33E+09
24	1.00E+08	3.00E+06	1.00E+08	6.77E+07±3.23E+07
48	2.00E+09	2.00E+06	4.00E+06	6.69E+08±6.66E+08
lactobacilli				
0	1.00E+05	1.00E+04	3.00E+05	1.37E+05±8.57E+04
24	5.00E+05	2.00E+05	1.00E+04	2.37E+05±1.43E+05
48	4.00E+04	3.00E+04	5.00E+03	2.50E+04±1.04E+04
Bacteroides spp.				
0	1.00E+10	1.10E+06	1.20E+07	3.34E+09±3.33E+09
24	2.00E+09	2.20E+07	1.00E+11	3.40E+10±3.30E+10
48	1.00E+06	3.60E+06	2.20E+08	7.49E+07±7.26E+07
Clostridium spp.				
0	2.10E+04	-	1.00E+04	1.55E+04±4.49E+03
24	-	1.00E+05	-	1.00E+05
48	-	-	1.00E+04	1.00E+04

Table C7. Counts of viable bacteria per g faeces following intravenous administration of amikacin to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
coliforms					
0	1.00E+06	2.00E+05	2.00E+05	2.00E+05	4.00E+05±2.00E+05
24	1.30E+04	1.00E+04	1.00E+05	1.00E+06	2.81E+05±2.41E+05
48	1.00E+04	1.00E+06	2.00E+05	2.00E+04	3.08E+05±2.35E+05
streptococci					
0	1.00E+06	6.00E+04	5.00E+06	1.80E+05	1.56E+06±1.17E+06
24	4.00E+04	4.00E+04	1.00E+04	2.00E+04	2.75E+04±7.50E+03
48	1.00E+05	1.00E+05	1.00E+05	1.00E+05	1.00E+05±0.00E+00
lactobacilli					
0	5.00E+04	2.00E+07	2.00E+10	3.00E+04	5.01E+09±5.00E+09
24	1.00E+06	1.00E+05	8.00E+04	3.00E+03	2.96E+05±2.36E+05
48	1.00E+07	3.00E+04	2.00E+06	1.00E+05	3.03E+06±8.83E+06
Bacteroides spp.					
0	1.00E+06	4.00E+07	1.10E+07	5.00E+06	1.43E+07±8.83E+06
24	1.80E+05	1.00E+08	1.60E+06	3.00E+06	2.62E+07±2.46E+07
48	7.00E+06	4.20E+09	2.10E+06	2.10E+10	6.30E+09±5.00E+09
Clostridium spp.					
0	1.00E+04	2.00E+04	1.00E+03	3.00E+04	1.53E+04±6.26E+03
24	1.00E+03	-	-	-	1.00E+03
48	2.00E+04	1.00E+04	1.00E+04	1.00E+03	1.03E+04±3.88E+03

Table C8. Counts of viable bacteria per g faeces following intravenous administration of amikacin to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
coliforms				
0	1.90E+04	2.00E+03	2.00E+04	1.37E+04±5.84E+03
24	1.00E+03	1.00E+04	5.00E+04	2.03E+04±1.51E+04
48	2.00E+04	1.00E+06	1.00E+04	3.43E+05±3.28E+05
streptococci				
0	4.00E+04	-	4.00E+03	2.20E+04±1.80E+04
24	1.00E+05	1.00E+03	7.00E+03	3.60E+04±3.20E+04
48	2.00E+03	2.00E+05	3.00E+03	6.83E+04±6.58E+04
lactobacilli				
0	6.00E+05	7.00E+04	4.00E+07	1.36E+07±1.32E+07
24	7.00E+05	1.30E+05	1.00E+05	3.10E+05±1.95E+05
48	4.00E+05	5.00E+04	2.00E+04	1.57E+05±1.22E+05
Bacteroides spp.				
0	9.50E+06	2.00E+09	1.00E+05	6.70E+08±6.65E+08
24	1.00E+06	2.20E+06	3.00E+05	1.17E+06±5.55E+05
48	1.00E+06	1.60E+05	3.20E+07	1.11E+07±1.05E+07
Clostridium spp.				
0	1.00E+07	-	-	1.00E+07
24	3.00E+05	-	-	3.00E+05
48	3.00E+05	-	-	3.00E+05

Table C9. Counts of viable bacteria per g faeces following intravenous administration of amikacin to donkeys

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	43.4	0.0	0.0	43.4
24	0.0	0.0	0.0	0.0	122.2	0.0	0.0	122.2
48	1.5	1.1	5.5	1.4	5.7	1.2	0.0	14.9

Table C10. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to horse 3

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	2.7
24	1.2	1.8	0.0	0.7	31.5	1.2	0.0	35.2
48	0.0	0.0	0.0	0.0	33.0	0.0	0.0	33.0

Table C11. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to horse 4

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.4	2.9	6.5	0.0	55.0	0.0	0.0	64.4
24	0.0	0.0	0.0	0.0	109.8	0.0	0.0	109.8
48	0.0	0.0	0.0	0.0	72.9	0.0	0.0	72.9

Table C12. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to horse 5

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C13. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony 7

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C14. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony 8

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C15. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony 9

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C16. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony 10

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	2.1	2.5	0.0	0.0	0.0	0.0	4.6
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	3.7	6.3	17.1	0.0	0.0	0.0	0.0	23.4

Table C17. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to donkey 14

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.9	4.9	1.9	0.0	71.6	0.0	0.0	78.4
24	5.6	9.0	6.7	0.0	81.6	0.0	0.0	97.3
48	2.5	1.9	8.2	0.0	41.2	0.0	0.0	51.3

Table C18. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to donkey 15

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.8	5.2	0.0	0.0	69.6	0.0	0.0	74.8
24	1.4	5.2	10.1	0.0	64.8	0.0	0.0	80.1
48	1.8	5.2	9.5	0.0	56.1	0.0	0.0	70.8

Table C19. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to donkey 16

	Horses		Ponies		Donkeys	
Time (h)	L	Total	L	Total	L	Total
0	0.8±0.8	36.8±18.11	0.0±0.0	0.0±0.0	1.2±0.6	52.6±24.0
24	0.4±0.4	89.1±27.2	0.0±0.0	0.0±0.0	2.3±1.7	59.1±30.0
48	0.5±0.5	40.3±17.1	0.0±0.0	0.0±0.0	2.7±0.6	48.5±13.8

Table D20. SCFA concentrations (mmol/kg) (mean±SEM) in faeces following intravenous administration of amikacin to horses, ponies and donkeys

Time (h)	Horse 3	Horse 4	Horse 5	mean±SEM
0	22.34	21.97	20.01	21.44±0.72
24	20.30	19.36	16.89	18.85±1.02
48	19.82	18.81	20.98	19.87±0.63

Table C21. Faecal dry matter content (%) following intravenous administration of amikacin to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
0	16.88	21.54	19.51	24.46	20.60±1.60
24	17.35	20.55	21.64	20.13	19.92±0.91
48	19.41	20.20	18.62	23.71	20.49±1.12

Table C22. Faecal dry matter content (%) following intravenous administration of amikacin to ponies

Time (h)	Donkey 14	Donkey 15	Donkey 16	mean±SEM
0	22.01	20.23	22.84	21.69±0.77
24	23.02	18.00	19.76	20.26±1.47
48	17.04	20.03	20.02	19.03±1.00

Table C23. Faecal dry matter content (%) following intravenous administration of amikacin to donkeys

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.033	58.27	57.93	56.99	55.73
0.083	43.12	49.87	43.97	54.46
0.25	42.04	39.86	38.85	47.35
0.5	35.59	36.15	30.40	33.88
0.75	29.15	22.59	30.79	23.55
1	23.52	21.55	22.93	22.53
1.5	22.29	20.27	20.59	18.81
2	16.11	18.09	18.25	16.82
4	8.42	11.99	8.67	10.41
6	3.83	7.81	3.59	5.51
8	2.18	3.92	2.05	4.14
12	0.84	1.90	0.42	1.24
24	0.00	0.04	0.00	0.00

Table C24. Plasma concentrations (μg/ml) of amikacin following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
coliforms				
0	1.00E+06	2.00E+04	1.00E+06	1.00E+05
24	2.00E+04	2.00E+05	2.00E+04	2.00E+04
48	1.00E+05	2.00E+05	1.00E+05	1.00E+05
streptococci				
0	4.00E+09	1.00E+06	1.00E+07	2.00E+08
24	1.00E+06	1.00E+05	2.00E+05	4.00E+05
48	1.00E+05	1.00E+04	5.00E+04	1.00E+05
lactobacilli				
0	1.00E+06	1.00E+08	1.00E+05	2.00E+06
24	1.50E+06	1.00E+06	1.00E+06	1.00E+07
48	2.00E+05	6.00E+04	1.00E+06	2.00E+06
Bacteroides spp.				
0	3.00E+10	1.00E+08	7.80E+10	1.00E+11
24	3.10E+10	1.10E+11	2.20E+10	1.50E+11
48	2.30E+08	1.70E+11	2.00E+09	1.40E+11
Clostridium spp.				
0	1.10E+05	1.00E+03	1.00E+04	2.00E+04
24	-	2.00E+03	4.00E+03	-
48	2.00E+04	1.00E+03	1.00E+03	-

Table C25. Counts of viable bacteria per ml caecal liquor following intravenous administration of amikacin to ponies

Time (h)	I1	I2	II1	II2
0	6.6	6.6	6.8	6.7
0.25	6.7	6.7	6.9	6.8
0.5	6.7	6.9	6.8	6.9
0.75	6.7	6.9	6.9	6.8
1	6.7	6.9	6.9	6.9
1.5	6.7	7.0	6.8	6.8
2	6.7	7.0	6.9	6.8
4	6.7	6.8	6.8	7.0
6	6.7	6.6	6.9	6.6
8	6.7	6.5	6.9	6.7
12	6.9	6.5	6.9	6.8
24	6.9	7.0	6.9	6.7
48	7.1	6.9	7.2	6.9

Table C26. Caecal liquor pH following intravenous administration of amikacin to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.1	31.5	12.9	0.9	12.0	2.9	2.4	62.6
0.25	1.9	28.8	12.0	0.0	16.7	1.2	0.0	58.7
0.5	1.9	27.5	10.2	0.5	10.5	1.0	0.0	49.7
0.75	0.9	24.3	10.0	0.0	10.6	0.0	0.0	44.9
1	1.7	22.6	9.5	0.3	9.3	0.8	0.7	43.2
1.5	1.9	22.5	9.8	0.0	13.2	0.0	0.0	45.5
2	2.9	24.8	11.7	0.0	15.4	0.8	0.7	53.4
4	8.9	27.2	17.9	0.0	56.0	0.0	0.0	101.1
6	5.7	27.0	13.0	0.0	25.3	0.0	0.0	65.3
8	3.6	27.3	14.2	0.0	24.0	0.4	0.0	65.9
12	2.2	28.4	12.2	0.0	16.5	0.0	0.0	57.1
24	1.0	19.8	9.6	0.0	13.5	0.0	0.0	42.9
48	2.0	16.3	10.2	0.0	8.4	0.6	0.0	35.5

Table C27a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of amikacin to pony I1

Time (h)	A	P	B	P+B
0	50.3	20.6	19.2	39.8
0.25	49.1	20.4	28.4	48.9
0.5	55.3	20.5	21.1	41.6
0.75	54.1	22.3	23.6	45.9
1	52.3	22.0	21.5	43.5
1.5	49.5	21.5	29.0	50.5
2	46.4	21.9	28.8	50.7
4	26.9	17.7	55.4	73.1
6	41.3	19.9	38.7	58.7
8	41.4	21.5	36.4	58.0
12	49.7	21.4	28.9	50.3
24	46.2	22.4	31.5	53.8
48	45.9	28.7	23.7	52.4

Table C27b. VFA concentrations (%) in caecal liquor following intravenous administration of amikacin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.7	26.1	7.6	0.6	11.4	1.9	0.0	47.6
0.25	0.7	25.5	8.5	0.2	8.7	0.4	0.0	43.3
0.5	1.3	21.5	7.9	0.4	9.1	0.6	0.0	39.5
0.75	0.6	21.7	8.1	0.0	10.5	0.5	1.6	42.4
1	1.4	23.1	9.6	0.7	13.2	1.2	2.2	50.0
1.5	0.7	23.4	7.5	0.0	24.9	0.0	0.0	55.8
2	2.1	21.9	7.7	0.6	20.6	1.0	1.8	53.6
4	1.1	35.1	10.8	0.6	9.3	0.8	0.0	56.6
6	2.0	12.8	5.2	1.2	8.0	1.9	0.0	29.1
8	1.1	28.9	8.6	0.0	19.1	0.9	0.0	57.5
12	3.3	50.5	12.1	0.0	68.7	0.0	0.0	131.3
24	1.1	21.8	8.7	0.0	12.4	0.0	0.0	42.9
48	1.9	26.1	9.7	0.6	20.2	1.1	0.0	57.7

Table C28a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of amikacin to pony I2

Time (h)	A	P	B	P+B
0	54.8	16.0	23.9	39.9
0.25	58.9	19.6	20.1	39.7
0.5	54.4	20.0	23.0	43.0
0.75	51.2	19.1	24.8	43.9
1	46.2	19.2	26.4	45.6
1.5	41.9	13.4	44.6	58.1
2	40.9	14.4	38.4	52.8
4	62.0	19.1	16.4	35.5
6	44.0	17.9	27.5	45.4
8	50.3	15.0	33.2	48.2
12	38.5	9.2	52.3	61.5
24	50.8	20.3	28.9	49.2
48	45.2	16.8	35.0	51.8

Table C28b. VFA concentrations (%) in caecal liquor following intravenous administration of amikacin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.0	37.4	13.7	1.6	8.5	2.1	0.0	63.3
0.25	1.6	33.0	12.5	1.6	9.6	3.1	0.0	59.8
0.5	0.2	34.5	13.3	1.0	9.6	1.3	0.0	59.7
0.75	1.4	35.3	14.0	1.4	11.9	3.0	0.0	65.6
1	0.5	36.1	12.9	0.8	10.3	3.5	0.0	63.6
1.5	1.5	33.6	13.3	1.8	11.5	2.5	0.0	62.7
2	0.5	34.5	13.6	1.3	12.3	2.1	1.3	65.1
4	3.6	38.1	16.1	1.7	18.4	4.0	1.8	80.1
6	0.9	31.2	13.4	1.2	12.6	1.8	0.0	60.2
8	3.0	37.5	15.9	2.2	14.0	3.6	1.7	74.9
12	1.0	36.9	15.4	0.6	12.0	4.6	0.0	69.5
24	2.0	32.2	12.9	1.2	13.5	3.1	1.3	64.2
48	0.7	33.1	13.6	0.0	12.0	0.0	0.0	58.7

Table C29a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of amikacin to pony II1

Time (h)	A	P	B	P+B
0	59.1	21.6	13.4	35.1
0.25	55.2	20.9	16.1	37.0
0.5	57.8	22.3	16.1	38.4
0.75	53.8	21.3	18.1	39.5
1	56.8	20.3	16.2	36.5
1.5	53.6	21.2	18.3	39.6
2	53.0	20.9	18.9	39.8
4	47.6	20.1	23.0	43.1
6	51.8	22.3	20.9	43.2
8	50.1	21.2	18.7	39.9
12	53.1	22.2	17.3	39.4
24	50.2	20.1	21.0	41.1
48	56.4	23.2	20.4	43.6

Table C29b. VFA concentrations (%) in caecal liquor following intravenous administration of amikacin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.0	51.7	15.5	0.7	11.3	0.0	0.0	79.2
0.25	2.4	45.1	13.5	1.7	11.8	0.0	0.0	72.1
0.5	1.1	43.0	12.2	0.0	11.9	0.0	0.0	67.1
0.75	1.5	32.9	10.6	0.5	7.7	0.0	0.0	51.7
1	1.9	40.6	13.9	0.0	11.5	0.0	0.0	66.0
1.5	1.5	34.8	12.0	1.9	12.5	1.3	0.0	62.5
2	0.0	32.5	10.8	0.9	8.2	0.0	0.0	52.4
4	0.5	19.4	7.4	0.0	41.6	1.4	0.0	69.8
6	3.3	39.3	10.9	2.3	29.4	2.3	3.4	87.6
8	0.4	32.9	10.5	0.9	28.6	1.2	0.0	74.1
12	1.3	52.9	15.4	0.9	23.9	1.5	0.0	94.6
24	2.4	40.1	15.7	2.3	15.6	0.0	0.0	73.7
48	0.0	51.6	15.7	0.0	16.2	0.0	0.0	83.5

Table C30a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of amikacin to pony II2

Time (h)	A	P	B	P+B
0	65.3	19.6	14.3	33.8
0.25	62.6	18.7	16.4	35.1
0.5	64.1	18.2	17.7	35.9
0.75	63.6	20.5	14.9	35.4
1	61.5	21.1	17.4	38.5
1.5	55.7	19.2	20.0	39.2
2	62.0	20.6	15.6	36.3
4	27.8	10.6	59.6	70.2
6	44.9	12.4	33.6	46.0
8	44.4	14.2	38.6	52.8
12	55.9	16.3	25.3	41.5
24	54.4	21.3	21.2	42.5
48	61.8	18.8	19.4	38.2

Table C30b. VFA concentrations (%) in caecal liquor following intravenous administration of amikacin to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.9	15.3	8.4	0.4	16.4	1.5	0.0	42.0
24	4.5	2.6	5.4	2.4	8.3	0.0	0.0	18.7
48	1.1	10.1	10.2	0.0	31.0	0.0	0.0	51.3

Table C31. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	1.5	1.0	0.0	10.9	0.0	0.0	13.4
24	0.0	2.2	0.0	0.0	40.5	0.0	0.0	42.7
48	0.0	1.0	0.0	0.0	9.1	1.4	0.0	11.5

Table C32. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	5.6	3.0	0.0	0.0	179.3	0.0	0.0	182.3
24	1.0	0.0	6.5	0.0	95.6	0.0	0.0	102.1
48	2.2	10.5	10.0	0.0	23.4	1.9	0.0	45.8

Table C33. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.7	2.2	3.4	1.6	17.3	1.3	0.0	25.8
24	0.0	2.2	0.0	0.0	29.4	0.0	0.0	31.6
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C34. SCFA concentrations (mmol/kg) in faeces following intravenous administration of amikacin to pony II2

Time (h)	I1	I2	II1	II2
0	22.89	18.63	22.82	19.66
24	25.09	22.66	26.66	23.04
48	24.33	23.79	22.16	20.46

Table C35. Faecal dry matter content (%) following intravenous administration of amikacin to ponies

	I1			I2		
Time (h)	0	24	48	0	24	120
urea (mmol/l)	3.2	4.2	4.0	2.4	3.2	2.4
sodium (mmol/l)	134	142	137	134	137	137
potassium (mmol/l)	4.2	1.3	2.8	3.9	3.2	4.2
chloride (mmol/l)	96	99	97	98	97	99
calcium (mmol/l)	2.92	2.80	2.98	3.35	2.84	3.05
magnesium (mmol/l)	0.58	0.52	0.69	0.80	0.60	0.59
phosphate (mmol/l)	0.75	1.12	0.93	1.05	1.32	1.07
creatinine (µmol/l)	117	117	128	123	128	121
bilirubin (µmol/l)	7	6	8	6	7	6
SAP (U/l)	270	276	290	273	270	264
AST (U/l)	266	290	317	311	322	293
GGT (U/l)	21	20	18	27	23	20
total protein (g/l)	69	75	77	77	76	69
albumin (g/l)	28	30	30	31	30	29
globulin (g/l)	41	45	47	46	46	40

Table C36. Plasma biochemistry following intravenous administration of amikacin to pony I

	II1			II2		
Time (h)	0	24	48	0	24	120
urea (mmol/l)	3.3	3.1	3.5	1.6	1.0	1.0
sodium (mmol/l)	134	139	137	134	132	136
potassium (mmol/l)	4.0	2.3	2.6	3.5	4.0	3.3
chloride (mmol/l)	99	100	101	97	99	97
calcium (mmol/l)	2.77	2.89	2.95	2.95	3.29	3.04
magnesium (mmol/l)	0.66	0.64	0.69	0.65	0.73	0.70
phosphate (mmol/l)	0.96	1.05	1.31	1.30	0.78	1.11
creatinine (µmol/l)	105	110	119	112	112	109
bilirubin (µmol/l)	8	9	10	11	11	9
SAP (U/l)	265	261	278	267	292	285
AST (U/l)	255	270	288	330	366	329
GGT (U/l)	25	20	22	28	28	29
total protein (g/l)	77	80	81	74	83	72
albumin (g/l)	28	29	31	28	32	29
globulin (g/l)	49	51	50	46	51	43

Table C37. Plasma biochemistry following intravenous administration of amikacin to pony II

	I1			I2		
Time (h)	0	24	48	0	24	120
WCC ($x10^9$/l)	7.0	8.1	6.1	7.6	7.0	6.8
RCC ($x10^{12}$/l)	6.16	6.28	5.92	8.04	7.05	6.10
Hb (g/dl)	10.8	11.5	10.8	13.4	12.0	10.9
Hct (l/l)	0.309	0.318	0.306	0.381	0.357	0.307
MCV (fl)	50	51	52	47	51	50
MCH (pg)	17.5	18.3	18.2	16.6	17.0	17.8
MCHC (g/dl)	34.9	36.1	35.2	35.1	33.6	35.5
PLTS (10^9/l)	100	114	108	104	122	102
MPV (fl)	6.4	6.3	6.3	6.2	6.0	6.2
PCT (%)	0.064	0.071	0.068	0.064	0.073	0.063
PDW	15.6	17.4	15.8	16.1	15.0	16.1
Neu (%)	46.9	42.0	32.5	32.5	35.0	36.0
Lym (%)	49.9	56.0	64.0	64.5	61.0	61.5
Mon (%)	2.6	1.0	1.5	2.0	2.0	1.5
Eos (%)	0.6	1.0	1.5	1.0	1.6	1.0
Bas (%)	0.0	0.0	0.5	0.0	0.4	0.0

Table C38. Haematology parameters following intravenous administration of amikacin to pony I

	II1			II2		
Time (h)	0	24	48	0	24	120
WCC (x10^9/l)	5.8	6.4	6.3	7.1	6.0	6.8
RCC (x10^{12}/l)	6.90	6.55	6.83	8.71	6.46	7.03
Hb (g/dl)	10.6	10.9	11.2	13.5	10.2	11.3
Hct (l/l)	0.312	0.300	0.315	0.387	0.297	0.323
MCV (fl)	45	46	46	44	46	46
MCH (pg)	15.3	16.6	16.3	15.4	15.7	16.0
MCHC (g/dl)	33.9	36.3	35.5	34.8	34.3	34.9
PLTS (10^9/l)	113	98	104	92	97	87
MPV (fl)	6.6	6.5	6.4	6.7	6.8	6.8
PCT (%)	0.074	0.063	0.066	0.061	0.065	0.059
PDW	15.1	16.9	17.1	16.4	16.1	17.6
Neu (%)	45.0	40.5	45.0	43.9	40.4	40.5
Lym (%)	51.5	54.6	48.5	49.9	59.6	53.0
Mon (%)	2.9	3.4	3.0	5.0	0.0	4.6
Eos (%)	0.5	0.9	3.0	0.6	0.0	0.4
Bas (%)	0.0	0.5	0.5	0.6	0.0	1.5

Table C39. Haematology parameters following intravenous administration of amikacin to pony II

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00
1	6.00	26.54	0.00	0.00
1.5	79.71	83.68	0.00	0.00
2	99.37	49.10	0.00	0.00
4	46.06	27.12	0.76	0.00
6	19.72	4.86	11.83	4.22
8	9.74	1.18	21.65	12.59
12	1.11	0.22	12.37	16.19
24	0.00	0.00	0.94	3.29
28	0.00	0.00	1.05	1.68
32	0.00	0.00	0.36	0.63
48	-	0.00	-	0.00

Table C40. Caecal liquor concentrations (µg/ml) of amikacin following oral administration to ponies

Time (h)	I1	I2	II1	II2	Pony 13
0	0.00	0.00	0.00	0.00	0.00
24	20.99	17.65	17.88	4.06	46.28
48	3.54	1.18	5.08	2.74	6.93
72	0.00	0.00	0.00	0.00	-
96	0.00	0.00	0.00	0.00	-

Table C41. Faecal concentrations (µg/g) of amikacin following oral administration to ponies

Time (h)	I1	I2	II1	II2	*Pony 13
coliforms					
0	1.00E+05	2.00E+05	2.00E+04	1.80E+04	1.00E+06
24	-	6.00E+04	2.00E+03	1.00E+03	2.10E+05
48	2.00E+04	2.00E+03	1.00E+04	4.00E+03	2.00E+06
72	2.00E+05	1.00E+07	1.00E+04	1.00E+07	7.00E+04
96	1.00E+09	1.00E+07	1.00E+04	1.00E+04	NS
168	2.00E+05	2.00E+03	1.80E+04	3.00E+03	NS
streptococci					
0	1.00E+04	9.00E+06	6.00E+04	3.00E+05	5.00E+04
24	2.00E+05	1.00E+07	2.00E+08	1.00E+05	2.00E+10
48	2.00E+07	1.00E+05	5.00E+05	1.00E+05	4.00E+10
72	6.00E+08	1.10E+05	2.00E+07	1.00E+05	3.00E+06
96	2.00E+07	5.00E+05	1.00E+05	4.00E+05	NS
168	9.00E+06	4.00E+04	3.00E+05	3.00E+06	NS
lactobacilli					
0	2.00E+05	3.00E+06	2.00E+05	2.00E+06	1.00E+06
24	1.00E+06	5.00E+08	3.00E+06	1.00E+06	3.00E+08
48	1.00E+05	1.00E+06	2.00E+05	4.00E+04	1.00E+09
72	1.00E+06	2.00E+05	1.00E+05	7.00E+05	3.00E+05
96	2.00E+06	2.00E+05	1.00E+07	2.00E+07	NS
168	3.00E+06	5.00E+07	2.00E+06	1.00E+05	NS
Bacteroides spp.					
0	1.30E+11	9.70E+07	3.80E+11	1.00E+10	1.30E+08
24	1.50E+11	1.60E+11	7.10E+10	4.10E+10	2.10E+10
48	1.30E+11	2.00E+08	2.20E+11	1.20E+10	1.40E+11
72	1.40E+11	1.50E+11	1.50E+11	1.90E+11	1.30E+11
96	2.60E+11	1.00E+10	1.40E+09	1.60E+11	NS
168	9.70E+07	8.10E+10	1.00E+10	2.60E+11	NS
Clostridium spp.					
0	1.60E+06	-	6.00E+06	1.10E+04	-
24	-	-	2.30E+05	3.00E+03	1.00E+03
48	1.00E+04	2.00E+04	1.00E+06	1.00E+05	1.00E+07
72	-	2.00E+03	1.00E+04	-	3.20E+05
96	1.00E+03	-	1.00E+04	1.00E+04	NS
168	-	-	1.10E+04	-	NS

Table C42. Counts of viable bacteria per ml caecal liquor or *per g faeces following oral administration of amikacin to ponies

Time (h)	I1	I2	II1	II2
0	6.9	6.8	6.9	6.9
0.25	6.8	6.9	6.8	6.8
0.5	6.9	6.8	6.8	6.8
0.75	6.9	6.8	6.8	6.7
1	6.8	6.7	6.8	6.8
1.5	6.9	6.8	6.8	6.7
2	6.9	6.4	6.9	6.3
4	6.7	6.4	6.8	6.4
6	6.5	5.8	6.7	6.3
8	6.5	6.3	6.7	6.7
12	6.4	6.4	6.5	6.7
24	7.0	6.8	6.7	7.0
28	6.5	6.4	6.6	6.8
32	6.6	6.5	7.4	6.8
48	6.9	6.6	6.8	6.8
52	6.7	6.6	6.7	6.7
56	6.6	6.6	6.6	6.7
72	6.9	7.2	6.8	7.2
96	6.7	6.8	6.7	6.8
168	6.8	7.0	6.9	6.9

Table C43. Caecal liquor pH following oral administration of amikacin to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.9	29.9	9.9	0.0	15.5	0.0	0.0	55.3
0.25	1.5	19.1	7.4	1.1	9.9	1.2	1.7	40.4
0.5	0.5	20.1	6.9	0.0	10.0	0.0	0.0	37.0
0.75	1.0	15.1	7.5	0.3	5.5	0.0	0.0	28.4
1	0.0	21.3	12.5	0.0	13.9	0.0	0.0	47.7
1.5	0.9	14.5	4.7	0.2	3.1	0.0	0.0	22.5
2	11.1	23.2	12.5	0.6	7.7	0.8	0.0	44.8
4	24.4	39.7	16.6	0.0	13.0	0.0	0.0	69.3
6	22.9	37.9	18.7	0.0	11.3	0.0	0.0	67.9
8	12.9	24.0	20.0	1.5	14.9	1.3	0.0	61.7
12	16.1	22.9	13.8	0.0	13.5	0.0	0.0	50.2
24	1.3	18.3	10.4	0.0	12.4	0.0	0.0	41.1
28	17.5	27.3	12.4	0.5	6.4	1.0	0.0	47.6
32	1.2	36.4	13.1	0.6	9.7	0.0	0.9	60.7
48	0.6	34.4	14.4	0.0	32.0	0.0	0.0	80.8
52	6.5	27.5	12.1	0.0	33.7	0.0	0.0	73.3
56	8.0	27.4	10.5	0.9	18.3	1.7	1.6	60.4
72	1.2	23.9	10.5	0.0	17.8	0.0	0.0	52.2
96	0.7	31.9	7.8	0.0	17.6	0.0	1.2	58.5
168	0.3	23.6	13.0	0.0	6.4	0.0	0.0	43.0

Table C44a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of amikacin to pony I1

Time (h)	A	P	B	P+B
0	54.1	17.9	28.0	45.9
0.25	47.3	18.3	24.5	42.8
0.5	54.3	18.6	27.0	45.7
0.75	53.2	26.4	19.4	45.8
1	44.7	26.2	29.1	55.3
1.5	64.4	20.9	13.8	34.7
2	51.8	27.9	17.2	45.1
4	57.3	24.0	18.8	42.7
6	55.8	27.5	16.6	44.2
8	38.9	32.4	24.1	56.6
12	45.6	27.5	26.9	54.4
24	44.5	25.3	30.2	55.5
28	57.4	26.1	13.4	39.5
32	60.0	21.6	16.0	37.6
48	42.6	17.8	39.6	57.4
52	37.5	16.5	46.0	62.5
56	45.4	17.4	30.3	47.7
72	45.8	20.1	34.1	54.2
96	54.5	13.3	30.1	43.4
168	54.9	30.2	14.9	45.1

Table C44b. VFA concentrations (%) in caecal liquor following oral administration of amikacin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.3	23.6	13.0	0.0	6.4	0.0	0.0	43.0
0.25	0.7	22.5	14.4	0.0	9.1	0.0	0.0	46.0
0.5	1.0	19.0	9.5	0.0	6.7	0.0	0.0	35.2
0.75	0.5	20.4	9.3	0.0	6.7	0.0	0.0	36.4
1	0.4	16.8	7.7	0.0	6.5	0.0	0.0	31.0
1.5	0.4	16.6	6.7	0.0	5.4	0.0	0.0	28.7
2	1.2	21.5	7.4	0.3	6.6	0.0	0.0	35.8
4	22.9	19.8	8.8	0.0	4.2	0.0	0.0	32.8
6	26.4	20.1	10.2	0.0	3.7	0.0	0.0	34.0
8	24.9	20.6	10.2	0.5	5.2	1.1	0.0	37.6
12	28.8	17.4	9.4	0.0	3.7	0.0	0.0	30.5
24	15.7	16.6	14.6	0.0	8.4	0.0	0.0	39.6
28	14.8	18.4	12.9	0.0	6.8	0.0	0.0	38.1
32	13.0	18.9	10.0	0.0	6.9	0.0	0.0	35.8
48	3.1	25.8	11.4	0.0	17.8	0.0	0.0	55.0
52	4.0	31.8	13.5	0.0	12.1	0.0	0.0	57.4
56	1.6	21.1	9.4	0.0	12.1	0.0	0.0	42.6
72	0.6	19.4	9.6	0.0	11.2	0.0	0.0	40.2
96	2.1	29.6	10.5	0.0	8.1	0.0	0.0	48.2
168	2.8	21.0	9.3	1.2	8.5	1.1	0.9	42.0

Table C45a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of amikacin to pony I2

Time (h)	A	P	B	P+B
0	54.9	30.2	14.9	45.1
0.25	48.9	31.3	19.8	51.1
0.5	54.0	27.0	19.0	46.0
0.75	56.0	25.5	18.4	44.0
1	54.2	24.8	21.0	45.8
1.5	57.8	23.3	18.8	42.2
2	60.1	20.7	18.4	39.1
4	60.4	26.8	12.8	39.6
6	59.1	30.0	10.9	40.9
8	54.8	27.1	13.8	41.0
12	57.0	30.8	12.1	43.0
24	41.9	36.9	21.2	58.1
28	48.3	33.9	17.8	51.7
32	52.8	27.9	19.3	47.2
48	46.9	20.7	32.4	53.1
52	55.4	23.5	21.1	44.6
56	49.5	22.1	28.4	50.5
72	48.3	23.9	27.9	51.7
96	61.4	21.8	16.8	38.6
168	50.0	22.1	20.2	42.4

Table C45b. VFA concentrations (%) in caecal liquor following oral administration of amikacin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	27.4	9.3	0.0	9.6	0.0	0.0	46.3
0.25	0.5	30.2	12.0	0.3	9.4	1.2	0.0	53.1
0.5	0.8	31.2	11.2	1.1	9.6	1.0	0.6	54.7
0.75	0.0	35.2	12.1	0.5	10.1	0.7	0.0	58.6
1	0.0	31.7	9.6	0.0	7.0	0.0	0.8	49.1
1.5	0.9	34.3	11.2	0.0	9.2	1.8	0.0	56.5
2	0.4	48.7	16.5	0.8	14.0	0.9	0.0	80.9
4	0.0	60.3	19.7	0.0	15.6	0.0	0.0	95.6
6	0.4	56.5	16.9	0.5	9.6	0.0	0.0	83.5
8	1.1	40.8	17.0	1.1	14.6	1.1	1.9	76.5
12	20.4	34.1	10.5	0.0	8.9	0.0	0.0	53.5
24	20.3	27.4	13.0	0.0	12.4	0.0	0.0	52.8
28	14.4	31.2	14.8	1.5	10.5	1.5	1.2	60.7
32	3.2	32.0	16.7	0.7	9.8	0.0	0.0	59.2
48	0.6	37.3	12.5	0.0	16.0	1.1	0.0	66.9
52	1.3	49.8	16.4	2.2	25.9	1.1	2.0	97.4
56	0.0	35.9	12.5	0.0	10.5	0.0	0.0	58.9
72	0.4	40.3	11.6	0.0	15.9	0.0	0.0	67.8
96	1.8	36.3	10.7	1.9	11.8	2.0	3.2	65.9
168	1.4	62.3	16.9	1.8	14.7	1.3	0.0	97.0

Table C46a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of amikacin to pony II1

Time (h)	A	P	B	P+B
0	59.2	20.1	20.7	40.8
0.25	56.9	22.6	17.7	40.3
0.5	57.0	20.5	17.6	38.0
0.75	60.1	20.6	17.2	37.9
1	64.6	19.6	14.3	33.8
1.5	60.7	19.8	16.3	36.1
2	60.2	20.4	17.3	37.7
4	63.1	20.6	16.3	36.9
6	67.7	20.2	11.5	31.7
8	53.3	22.2	19.1	41.3
12	63.7	19.6	16.6	36.3
24	51.9	24.6	23.5	48.1
28	51.4	24.4	17.3	41.7
32	54.1	28.2	16.6	44.8
48	55.8	18.7	23.9	42.6
52	51.1	16.8	26.6	43.4
56	61.0	21.2	17.8	39.0
72	59.4	17.1	23.5	40.6
96	55.1	16.2	17.9	34.1
168	64.2	17.4	15.2	32.6

Table C46b. VFA concentrations (%) in caecal liquor following oral administration of amikacin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.4	62.3	16.9	1.8	14.7	1.3	0.0	97.0
0.25	1.4	35.7	10.1	1.6	5.8	1.6	0.0	54.8
0.5	0.3	39.5	10.1	0.9	6.9	0.0	0.0	57.4
0.75	0.0	32.3	9.2	0.7	3.9	0.0	0.0	46.1
1	0.8	35.8	8.8	0.0	4.7	0.0	0.0	49.3
1.5	0.0	47.1	11.5	0.5	6.5	0.0	0.0	65.6
2	1.6	33.7	10.8	2.2	7.2	1.8	2.2	57.9
4	0.5	33.2	9.2	0.0	4.7	0.0	0.0	47.1
6	0.0	36.1	9.3	0.0	5.1	0.0	0.0	50.5
8	1.2	41.3	14.6	1.5	6.4	1.6	0.0	65.4
12	0.6	41.5	18.3	0.0	7.2	0.0	0.0	67.0
24	7.8	31.8	12.3	0.0	4.4	0.6	0.0	49.1
28	5.2	35.6	16.2	1.6	5.6	1.7	2.9	63.6
32	0.6	29.9	12.9	0.3	4.8	0.0	0.0	47.9
48	0.8	38.8	14.1	0.3	8.4	0.0	0.0	61.6
52	1.5	34.8	13.3	1.3	7.8	0.0	0.0	57.2
56	5.8	35.3	13.5	0.0	10.0	0.0	0.0	58.8
72	2.3	34.9	15.2	0.0	9.1	0.0	0.0	59.2
96	1.1	33.4	15.0	0.7	8.9	0.8	0.0	58.8
168	0.4	28.8	8.5	0.5	5.4	0.6	0.0	43.8

Table C47a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of amikacin to pony II2

Time (h)	A	P	B	P+B
0	64.2	17.4	15.2	32.6
0.25	65.1	18.4	10.6	29.0
0.5	68.8	17.6	12.0	29.6
0.75	70.1	20.0	8.5	28.4
1	72.6	17.8	9.5	27.4
1.5	71.8	17.5	9.9	27.4
2	58.2	18.7	12.4	31.1
4	70.5	19.5	10.0	29.5
6	71.5	18.4	10.1	28.5
8	63.1	22.3	9.8	32.1
12	61.9	27.3	10.7	38.1
24	64.8	25.1	9.0	34.0
28	56.0	25.5	8.8	34.3
32	62.4	26.9	10.0	37.0
48	63.0	22.9	13.6	36.5
52	60.8	23.3	13.6	36.9
56	60.0	23.0	17.0	40.0
72	59.0	25.7	15.4	41.0
96	56.8	25.5	15.1	40.6
168	65.8	19.4	12.3	31.7

Table C47b. VFA concentrations (%) in caecal liquor following oral administration of amikacin to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.8	5.9	2.8	1.1	27.1	1.7	0.0	38.6
24	3.2	2.4	9.1	0.0	31.3	0.0	0.0	42.8
48	3.2	1.5	0.0	0.0	20.2	0.0	0.0	21.7
72	0.0	0.0	5.7	0.0	37.4	0.0	0.0	43.1
96	2.3	0.0	0.0	0.0	49.5	0.0	0.0	49.5
168	0.0	3.1	3.4	0.0	30.0	0.0	0.0	36.5

Table C48. SCFA concentrations (mmol/kg) in faeces following oral administration of amikacin to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	3.1	3.4	0.0	30.0	0.0	0.0	36.5
24	0.0	4.1	7.9	0.0	13.8	0.0	0.0	25.8
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	1.4	6.2	7.9	0.0	0.0	0.0	0.0	14.1
96	0.0	11.4	7.9	0.0	25.4	0.0	0.0	44.7
168	2.2	0.0	5.9	0.0	14.1	0.0	0.0	20.0

Table C49. SCFA concentrations (mmol/kg) in faeces following oral administration of amikacin to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	15.4	0.0	62.2	0.0	74.9	0.0	0.0	137.1
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	3.1	3.5	0.0	83.3	0.0	0.0	89.9
168	0.0	2.4	3.9	0.0	36.5	0.0	0.0	42.8

Table C50. SCFA concentrations (mmol/kg) in faeces following oral administration of amikacin to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	2.4	3.9	0.0	36.5	0.0	0.0	42.8
24	0.0	4.0	0.0	0.0	13.0	0.0	0.0	17.0
48	0.0	0.0	4.1	0.0	89.9	0.0	0.0	94.0
72	3.8	15.2	7.2	0.0	20.5	0.0	0.0	42.9
96	2.1	33.0	14.4	0.0	55.2	0.0	0.0	102.6
168	2.5	5.3	8.1	0.0	40.6	0.0	0.0	54.0

Table C51. SCFA concentrations (mmol/kg) in faeces following oral administration of amikacin to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table C52. SCFA concentrations (mmol/kg) in faeces following oral administration of amikacin to pony 13

Time (h)	I1	I2	II1	II2	Pony 13
0	22.02	23.44	21.48	22.94	17.75
24	22.32	18.88	18.79	19.75	19.39
48	22.05	19.13	17.39	19.95	23.76
72	19.43	21.64	19.47	20.82	19.07
96	19.55	20.42	18.99	22.23	NS
168	23.44	20.21	22.94	20.11	NS

Table C53. Faecal dry matter content (%) following oral administration of amikacin to ponies

Time (h)	0	24	48	168
urea (mmol/l)	2.4	2.6	1.8	2.1
sodium (mmol/l)	137	136	134	136
potassium (mmol/l)	4.2	3.4	4.1	3.5
chloride (mmol/l)	99	100	98	99
calcium (mmol/l)	3.05	3.06	2.98	3.03
magnesium (mmol/l)	0.59	0.74	0.64	0.66
phosphate (mmol/l)	1.07	0.99	1.17	1.07
creatinine (µmol/l)	121	142	134	135
bilirubin (µmol/l)	6	6	6	5
SAP (U/l)	264	287	278	270
AST (U/l)	293	325	292	288
GGT (U/l)	20	22	19	21
total protein (g/l)	69	76	68	66
albumin (g/l)	29	30	30	27
globulin (g/l)	40	46	38	39

Table C54. Plasma biochemistry following oral administration of amikacin to pony I1

Time (h)	0	24	48	168
urea (mmol/l)	2.1	2.5	1.8	2.4
sodium (mmol/l)	136	133	135	135
potassium (mmol/l)	3.5	3.9	4.2	4.0
chloride (mmol/l)	99	106	103	95
calcium (mmol/l)	3.03	3.08	3.22	2.89
magnesium (mmol/l)	0.66	0.69	0.71	0.63
phosphate (mmol/l)	1.07	1.41	1.18	1.11
creatinine (µmol/l)	135	133	127	133
bilirubin (µmol/l)	5	6	6	6
SAP (U/l)	270	294	309	276
AST (U/l)	288	308	326	289
GGT (U/l)	21	26	25	20
total protein (g/l)	66	69	71	62
albumin (g/l)	27	29	29	25
globulin (g/l)	39	40	42	37

Table C55. Plasma biochemistry following oral administration of amikacin to pony I2

Time (h)	0	24	48	168
urea (mmol/l)	1.0	1.2	1.0	0.5
sodium (mmol/l)	136	138	135	136
potassium (mmol/l)	3.3	2.5	3.2	3.3
chloride (mmol/l)	97	108	103	102
calcium (mmol/l)	3.04	3.14	2.77	3.06
magnesium (mmol/l)	0.70	0.80	0.67	0.71
phosphate (mmol/l)	1.11	1.84	1.88	0.98
creatinine (µmol/l)	109	123	118	124
bilirubin (µmol/l)	9	9	10	9
SAP (U/l)	285	317	307	296
AST (U/l)	329	356	343	345
GGT (U/l)	29	22	17	21
total protein (g/l)	72	79	73	72
albumin (g/l)	29	29	31	29
globulin (g/l)	43	50	42	43

Table C56. Plasma biochemistry following oral administration of amikacin to pony II1

Time (h)	0	24	48	168
urea (mmol/l)	0.5	0.7	0.4	0.6
sodium (mmol/l)	136	134	136	134
potassium (mmol/l)	3.3	3.6	3.9	3.2
chloride (mmol/l)	102	104	105	100
calcium (mmol/l)	3.06	3.04	2.98	2.99
magnesium (mmol/l)	0.71	0.71	0.73	0.65
phosphate (mmol/l)	0.98	1.01	1.21	0.97
creatinine (µmol/l)	124	124	122	116
bilirubin (µmol/l)	9	9	10	8
SAP (U/l)	296	302	316	321
AST (U/l)	345	350	383	353
GGT (U/l)	21	20	23	28
total protein (g/l)	72	73	70	68
albumin (g/l)	29	30	28	28
globulin (g/l)	43	43	42	40

Table C57. Plasma biochemistry following oral administration of amikacin to pony II2

Time (h)	0	24	48	72	168
WCC ($x10^9$/l)	6.8	6.4	6.7	6.9	6.2
RCC ($x10^{12}$/l)	6.10	6.97	6.07	6.22	5.57
Hb (g/dl)	10.9	12.2	10.9	10.9	9.3
Hct (l/l)	0.307	0.355	0.307	0.316	0.281
MCV (fl)	50	51	51	51	50
MCH (pg)	17.8	17.5	17.9	17.5	16.6
MCHC (g/dl)	35.5	34.3	35.5	34.4	33.0
PLTS (10^9/l)	102	121	103	115	102
MPV (fl)	6.2	5.4	5.9	5.7	6.1
PCT (%)	0.063	0.065	0.060	0.065	0.062
PDW	16.1	20.3	16.9	15.7	16.3
Neu (%)	36.0	40.0	39.5	38.0	42.6
Lym (%)	61.5	56.0	59.0	59.0	55.0
Mon (%)	1.5	3.4	0.5	1.0	0.5
Eos (%)	1.0	0.5	1.0	2.0	1.4
Bas (%)	0.0	0.0	0.0	0.0	0.5

Table C58. Haematology parameters following oral administration of amikacin to pony I1

Time (h)	0	24	48	72	168
WCC (x10^9/l)	6.2	6.1	7.3	6.1	6.9
RCC (x10^{12}/l)	5.57	5.83	6.64	6.04	5.74
Hb (g/dl)	9.3	10.3	11.7	10.5	9.8
Hct (l/l)	0.281	0.297	0.332	0.304	0.291
MCV (fl)	50	51	50	50	51
MCH (pg)	16.6	17.6	17.6	17.3	17.0
MCHC (g/dl)	33.0	34.6	35.2	34.5	33.6
PLTS (10^9/l)	102	101	108	113	80
MPV (fl)	6.1	5.8	5.7	5.6	5.8
PCT (%)	0.062	0.058	0.061	0.063	0.046
PDW	16.3	15.5	15.7	16.0	18.9
Neu (%)	42.6	36.0	31.0	41.0	33.0
Lym (%)	55.0	61.0	62.0	54.6	63.0
Mon (%)	0.5	1.0	3.0	3.9	2.5
Eos (%)	1.4	2.0	4.0	0.5	1.0
Bas (%)	0.5	0.0	0.0	0.0	0.4

Table C59. Haematology parameters following oral administration of amikacin to pony I2

Time (h)	0	24	48	72	168
WCC (x10^9/l)	6.8	6.6	6.4	6.8	6.4
RCC (x10^{12}/l)	7.03	7.03	6.67	6.79	6.58
Hb (g/dl)	11.3	11.3	10.6	10.7	10.3
Hct (l/l)	0.323	0.320	0.304	0.310	0.304
MCV (fl)	46	46	46	46	46
MCH (pg)	16.0	16.0	15.8	15.7	15.6
MCHC (g/dl)	34.9	35.3	34.8	34.5	33.8
PLTS (10^9/l)	87	102	103	101	95
MPV (fl)	6.8	6.2	6.7	6.3	6.5
PCT (%)	0.059	0.063	0.069	0.063	0.061
PDW	17.6	17.7	17.9	17.4	16.9
Neu (%)	40.5	41.0	36.0	42.0	43.0
Lym (%)	53.0	52.9	60.6	54.0	54.0
Mon (%)	4.6	3.5	0.9	3.0	3.0
Eos (%)	0.4	2.6	2.5	1.0	0.0
Bas (%)	1.5	0.0	0.0	0.0	0.0

Table C60. Haematology parameters following oral administration of amikacin to pony II1

Time (h)	0	24	48	72	168
WCC (x10^9/l)	6.4	6.3	6.0	6.1	6.2
RCC (x10^{12}/l)	6.58	6.76	6.67	6.67	6.37
Hb (g/dl)	10.3	10.6	10.5	10.4	10.0
Hct (l/l)	0.304	0.313	0.307	0.308	0.297
MCV (fl)	46	46	46	46	47
MCH (pg)	15.6	15.6	15.7	15.5	15.6
MCHC (g/dl)	33.8	33.8	34.2	33.7	33.6
PLTS (10^9/l)	95	101	97	94	90
MPV (fl)	6.5	6.3	6.4	6.3	6.2
PCT (%)	0.061	0.063	0.062	0.059	0.055
PDW	16.9	15.8	17.1	17.4	16.1
Neu (%)	43.0	43.0	45.0	41.0	32.6
Lym (%)	54.0	51.9	52.0	51.0	61.9
Mon (%)	3.0	2.0	2.0	5.0	5.0
Eos (%)	0.0	2.0	1.0	3.0	0.5
Bas (%)	0.0	1.0	0.0	0.0	0.0

Table C61. Haematology parameters following oral administration of amikacin to pony II2

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.12	0.18	0.00	0.00	0.08±0.05
1	1.10	1.62	0.86	0.38	0.99±0.26
5	6.51	8.78	4.34	4.98	6.15±0.99
10	13.35	12.50	6.02	8.55	10.11±1.72
20	16.63	25.83	10.79	19.94	18.30±3.14
40	30.18	50.45	10.30	28.35	29.82±8.21
80	49.10	62.96	19.66	46.33	44.51±9.05

Table C62a. Concentrations (µg/ml) of amikacin in caecal liquor following incubation *in vitro* for 3 h

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.00	0.00±0.00
1	0.52	0.93	0.56	0.50	0.63±0.10
5	5.52	5.71	6.42	5.08	5.68±0.28
10	11.65	9.46	12.92	9.49	10.88±0.85
20	18.68	20.94	22.88	14.5	19.25±1.80
40	24.41	31.29	41.45	21.28	29.61±4.47
80	40.15	39.00	65.99	29.56	43.68±7.81

Table C62b. Concentrations (µg/ml) of amikacin in caecal liquor following incubation *in vitro* for 24 h

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.8	27.9	10.9	1.7	6.0	0.0	0.0	46.5
0.25	0.3	28.0	11.0	0.0	6.5	1.0	0.0	46.6
1	1.2	31.5	12.3	0.8	6.5	0.0	0.0	51.1
5	0.3	24.4	10.5	0.0	5.1	0.6	0.0	40.6
10	3.6	28.2	11.8	0.0	4.9	1.5	0.0	46.4
20	3.9	28.1	10.7	1.0	7.1	1.8	0.0	48.7
40	3.5	28.8	12.6	0.9	5.8	0.0	0.0	48.0
80	5.7	28.2	11.0	1.3	8.2	2.2	0.0	50.8

Table C63a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 3 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	2.5	29.8	10.8	0.4	4.4	1.2	0.0	46.6
0.25	3.3	30.1	12.6	1.2	5.2	1.5	2.6	53.3
1	2.6	31.9	12.6	0.3	4.8	0.5	0.0	50.1
5	0.9	27.2	10.3	0.9	4.7	1.6	0.0	44.7
10	0.0	34.6	12.0	0.0	6.3	0.0	0.0	52.9
20	0.6	34.6	13.4	1.0	6.4	2.1	0.0	57.6
40	0.0	26.8	10.8	0.6	4.1	0.0	0.0	42.3
80	1.2	24.3	10.5	2.0	5.0	1.5	0.0	43.3

Table C63b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 24 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	29.5	8.0	0.0	1.9	0.0	0.0	39.4
0.25	0.0	31.9	8.9	0.0	1.7	0.0	0.0	42.6
1	0.0	31.1	14.8	0.3	1.5	0.0	0.0	47.6
5	0.0	34.8	9.9	0.0	2.3	0.0	0.0	47.0
10	0.0	33.4	9.6	0.0	8.0	0.6	0.0	51.6
20	0.0	39.1	12.2	0.0	6.9	0.0	0.0	58.2
40	0.1	40.6	12.8	0.0	8.0	0.0	0.0	61.4
80	0.0	37.8	10.5	0.0	8.3	0.0	0.0	56.7

Table C64a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 3 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	35.2	9.3	0.0	3.0	0.5	0.0	48.0
0.25	0.0	36.7	10.4	0.0	4.5	0.0	0.0	51.7
1	0.0	38.0	11.3	0.6	4.5	0.0	0.0	54.4
5	0.0	39.1	9.9	0.0	3.3	0.0	0.0	52.3
10	0.0	38.8	11.1	0.0	5.1	0.2	0.0	55.2
20	0.0	39.4	13.0	0.0	4.1	0.0	0.0	56.6
40	0.0	38.4	12.3	0.4	4.6	0.3	0.0	55.9
80	0.0	41.8	13.3	0.0	13.2	0.0	0.0	68.3

Table C64b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 24 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.2	45.5	15.3	0.1	7.4	1.2	0.6	70.1
0.25	0.0	41.8	12.5	0.0	6.1	1.6	0.0	62.0
1	0.0	42.6	14.1	0.0	6.5	0.6	0.9	64.7
5	0.0	44.9	15.9	0.0	5.7	1.7	0.0	68.3
10	0.4	44.7	18.9	0.3	6.9	1.6	0.6	73.2
20	0.0	43.8	14.4	0.0	5.9	2.2	0.0	66.3
40	0.1	48.8	17.5	0.0	7.9	0.0	0.0	74.1
80	0.0	52.0	16.2	0.0	10.0	2.0	0.0	80.2

Table C65a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 3 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.1	46.2	13.2	0.0	6.8	1.0	0.0	67.2
0.25	0.0	48.3	13.7	0.0	6.5	4.7	0.0	73.2
1	0.9	44.6	14.0	0.2	7.2	2.1	1.0	69.1
5	0.3	50.3	12.8	0.0	6.8	0.9	1.1	71.9
10	0.0	48.4	11.9	0.0	5.9	0.3	0.0	66.5
20	0.2	45.1	11.8	0.0	6.6	1.6	1.3	66.3
40	0.0	55.1	14.4	0.0	6.6	0.6	0.4	77.1
80	0.0	47.0	12.7	0.0	6.2	0.8	0.5	67.2

Table C65b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 24 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	52.3	14.0	0.0	5.8	0.9	0.0	73.0
0.25	0.0	43.8	11.0	0.0	4.2	0.2	0.0	59.2
1	0.0	47.1	12.1	0.0	6.2	1.2	0.0	66.6
5	0.0	52.1	14.3	0.0	7.8	0.0	0.0	74.2
10	0.3	46.6	13.5	0.1	6.8	1.2	1.2	69.4
20	0.0	43.8	13.4	0.3	7.6	0.6	0.0	65.7
40	0.1	44.3	14.6	0.0	8.5	1.4	2.0	70.8
80	0.0	47.2	13.0	0.0	6.8	0.0	0.0	67.0

Table C66a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 3 h (replicate 4)

Conc. (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.6	34.0	7.9	1.0	6.2	1.7	0.8	51.6
0.25	0.0	37.4	12.0	0.0	6.4	1.1	0.4	57.4
1	1.5	38.8	11.0	1.2	8.0	1.9	1.6	62.5
5	0.0	41.5	10.9	0.7	7.3	0.9	0.0	61.3
10	0.8	36.2	9.2	1.1	6.1	1.4	0.0	53.9
20	0.0	38.0	10.9	0.9	6.7	1.4	0.0	57.9
40	1.3	35.1	9.5	0.6	5.6	0.0	0.0	50.9
80	0.5	41.5	10.3	0.0	4.8	1.8	0.0	58.5

Table C66b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with amikacin for 24 h (replicate 4)

	3 h		24 h	
Conc (µg/ml)	L	Total	L	Total
0	0.8±0.5	57.3±8.4	0.8±0.6	53.4±4.7
0.25	0.1±0.1	52.6±4.7	0.8±0.8	58.9±4.9
1	0.3±0.3	57.5±4.8	1.3±0.5	59.0±4.3
5	0.1±0.1	57.5±8.1	0.3±0.2	57.6±5.9
10	1.1±0.8	60.1±6.6	0.2±0.2	57.1±3.2
20	1.0±1.0	59.7±4.1	0.2±0.1	59.6±2.3
40	1.0±0.8	63.6±5.8	0.3±0.3	56.5±7.4
80	1.4±1.4	63.7±6.4	0.4±0.3	59.3±5.8

Table C67. SCFA concentrations (mmol/l) (mean±SEM) in caecal liquor following *in vitro* incubation with amikacin

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
1	0.81	0.78	1.04	0.86	0.87±0.06
2	2.49	2.08	1.65	1.76	2.00±0.19
5	3.81	4.33	4.38	4.39	4.23±0.14
10	6.99	7.23	7.20	6.92	7.09±0.08

Table C68. Concentrations (µg/ml) (mean±SEM) of amikacin following incubation *in vitro* at pH 1.9 for 1 h

Appendix D - Oxytetracycline

Time (h)	Horse 1	Horse 2	Horse 3	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.083	69.99	84.73	91.61	82.11±6.38
0.25	40.46	45.18	40.16	41.93±1.63
0.5	24.62	30.64	23.75	26.34±2.17
0.75	22.3	22.92	22.43	22.55±0.19
1	19.13	21.07	16.43	18.88±1.35
1.5	15.46	17.06	14.59	15.70±0.72
2	13.49	14.22	11.33	13.01±0.87
4	8.11	9.88	8.10	8.70±0.59
8	7.26	7.43	5.86	6.85±0.50
12	4.84	4.97	4.63	4.81±0.10
24	2.11	2.67	2.24	2.34±0.17
36	1.15	1.15	1.03	1.11±0.04
48	0.55	0.52	0.48	0.52±0.02
72	0.25	0.19	0.16	0.20±0.03
96	0.16	0.00	0.10	0.09±0.05

Table D1. Plasma concentrations (μg/ml) of oxytetracycline following intravenous administration to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.083	50.37	74.99	24.71	48.72	49.70±10.27
0.25	30.32	42.88	16.12	32.63	30.49±5.51
0.5	18.88	25.72	12.35	24.51	20.37±3.06
0.75	16.97	22.12	9.66	17.34	16.52±2.57
1	15.52	19.21	7.69	15.15	14.39±2.41
1.5	11.82	15.31	6.36	12.13	11.41±1.86
2	10.19	12.8	5.47	10.19	9.66±1.53
4	6.29	8.12	3.57	6.28	6.07±0.94
8	3.46	5.87	2.73	4.30	4.09±0.67
12	2.33	4.5	2.64	2.84	3.08±0.49
24	1.54	2.99	1.13	1.37	1.76±0.42
28	1.32	NS	0.85	NS	1.09±0.24
32	0.95	NS	0.83	NS	0.89±0.06
36	0.91	2.4	0.64	0.60	1.14±0.43
48	0.42	0.99	0.33	0.33	0.52±0.16
72	0.22	0.39	0.25	0.11	0.24±0.06
96	0.00	0.00	0.00	0.00	0.00±0.00

Table D2. Plasma concentrations (μg/ml) of oxytetracycline following intravenous administration to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
0.083	57.77	47.96	64.77	56.83±4.88
0.25	35.69	26.71	43.30	35.23±4.79
0.5	30.83	14.45	33.64	26.31±5.98
0.75	22.45	11.48	20.70	18.21±3.40
1	19.99	9.51	19.12	16.21±3.36
1.5	14.96	7.55	11.66	11.39±2.14
2	11.59	6.94	9.01	9.18±1.35
4	6.03	3.82	4.94	4.93±0.64
8	2.66	2.15	2.24	2.35±0.16
12	1.58	1.15	1.32	1.35±0.13
24	0.27	1.1	0.32	0.56±0.27
36	0.00	0.54	0.12	0.22±0.16
48	0.00	0.22	0.00	0.07±0.07
72	0.00	0.00	0.00	0.00±0.00

Table D3. Plasma concentrations (μg/ml) of oxytetracycline following intravenous administration to donkeys

Parameter	Horse 1	Horse 2	Horse 3
t1/2 B2 (h)	0.19	0.20	0.11
t1/2 B1 (h)	13.13	10.38	11.89
Cp0 (μg/ml)	87.25	99.89	146.41
Vc (ml/kg)	114.60	100.11	68.30
AUCobs (μg.h/ml)	216.36	230.64	204.50
AUMCobs (μg.h^2/ml)	3344.43	2945.05	2846.92
AUC (μg.h/ml)	255.89	260.23	244.41
AUMC (μg.h^2/ml)	4463.10	3548.48	3836.83
MRT (h)	15.46	12.77	13.92
Vdarea (ml/kg)	740.40	575.78	702.19
Vdss (ml/kg)	681.61	524.00	642.31
CLb (ml/h.kg)	39.08	38.43	40.42
kel (/h)	0.34	0.38	0.60
k21 (/h)	0.56	0.61	0.62
k12 (/h)	2.78	2.60	5.18

Table D4. Disposition kinetics of oxytetracycline following intravenous administration to horses

Parameter	Pony 7	Pony 8	Pony 9	Pony 10
t1/2 B2 (h)	0.20	0.18	0.29	0.30
t1/2 B1 (h)	11.36	13.30	13.17	9.93
Cp0 (μg/ml)	61.86	94.63	27.30	54.24
Vc (ml/kg)	161.66	105.67	366.30	184.35
AUCobs (μg.h/ml)	141.49	240.77	101.56	141.08
AUMCobs (μg.h^2/ml)	2027.97	4205.87	1729.36	1633.53
AUC (μg.h/ml)	166.92	280.33	113.29	168.86
AUMC (μg.h^2/ml)	2485.98	4972.68	1981.88	2155.39
MRT (h)	14.33	17.47	17.03	11.58
Vdarea (ml/kg)	982.05	684.45	1677.69	848.72
Vdss (ml/kg)	892.26	632.77	1544.09	755.93
CLb (ml/h.kg)	59.91	35.67	88.27	59.22
kel (/h)	0.37	0.34	0.24	0.32
k21 (/h)	0.56	0.58	0.52	0.50
k12 (/h)	2.52	2.91	1.66	1.55

Table D5. Disposition kinetics of oxytetracycline following intravenous administration to ponies

Parameter	Donkey 15	Donkey 16	Donkey 17
t1/2 B2 (h)	0.31	0.16	0.30
t1/2 B1 (h)	3.84	8.79	5.50
Cp0 (μg/ml)	60.32	66.14	74.84
Vc (ml/kg)	165.78	151.19	133.63
AUCobs (μg.h/ml)	104.56	89.71	97.70
AUMCobs (μg.h^2/ml)	445.34	997.86	451.39
AUC (μg.h/ml)	110.15	110.26	107.95
AUMC (μg.h^2/ml)	510.50	1235.10	595.62
MRT (h)	4.26	11.12	4.62
Vdarea (ml/kg)	503.75	1150.47	675.33
Vdss (ml/kg)	420.78	1015.92	511.11
CLb (ml/h.kg)	90.79	90.69	92.63
kel (/h)	0.55	0.60	0.69
k21 (/h)	0.73	0.58	0.45
k12 (/h)	1.13	3.35	1.28

Table D6. Disposition kinetics of oxytetracycline following intravenous administration to donkeys

Time (h)	Horse 1	Horse 2	Horse 3	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
24	7.58	12.04	5.91	8.51±1.83
48	6.96	7.23	3.76	5.98±1.11
72	0.76	2.65	1.33	1.58±0.56
96	0.72	0.91	0.54	0.72±0.11
168	0.00	0.00	0.00	0.00±0.00

Table D7. Faecal concentrations (μg/g) of oxytetracycline following intravenous administration to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
24	3.37	9.54	9.39	4.16	7.43±2.03
48	3.10	5.17	4.07	3.65	4.11±0.60
72	0.71	0.88	2.45	1.44	1.35±0.55
168	0.00	0.00	0.00	0.00	0.00±0.00

Table D8. Faecal concentrations (μg/g) of oxytetracycline following intravenous administration to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
0	0.00	0.00	0.00	0.00±0.00
24	10.69	5.98	13.16	9.94±2.11
48	5.00	4.24	1.83	3.69±0.96
72	1.61	1.01	0.67	1.10±0.27
96	0.96	0.80	0.58	0.78±0.11
168	0.00	0.00	0.00	0.00±0.00

Table D9. Faecal concentrations (μg/g) of oxytetracycline following intravenous administration to donkeys

Time (h)	Horse 1	Horse 2	Horse 3	mean±SEM
coliforms				
0	1.30E+06	1.00E+06	2.00E+04	7.73E+05±3.86E+05
24	2.00E+07	1.10E+05	1.90E+04	6.71E+06±6.65E+06
48	1.20E+06	2.50E+05	1.00E+06	8.17E+05±2.89E+05
72	1.00E+06	1.00E+05	1.00E+08	3.37E+07±3.32E+07
96	3.00E+05	4.00E+05	1.00E+05	2.67E+05±8.82E+04
168	1.50E+06	1.00E+05	2.00E+05	6.00E+05±4.51E+05
streptococci				
0	1.00E+05	1.00E+04	7.00E+07	2.34E+07±2.33E+07
24	1.00E+06	2.00E+04	9.00E+05	6.40E+05±3.11E+05
48	3.00E+04	1.00E+03	1.00E+08	3.33E+07±3.33E+07
72	2.00E+05	1.00E+04	1.00E+08	3.34E+07±3.33E+07
96	6.00E+07	2.00E+05	2.00E+10	6.69E+09±6.66E+09
168	5.00E+07	1.00E+05	2.00E+08	8.34E+07±6.01E+07
lactobacilli				
0	2.00E+06	2.00E+04	2.00E+08	6.73E+07±6.63E+07
24	1.00E+06	2.00E+04	8.00E+04	3.67E+05±3.17E+05
48	4.30E+11	1.00E+07	1.00E+08	1.43E+11±1.43E+11
72	3.00E+05	1.00E+05	2.00E+08	6.68E+07±6.66E+07
96	1.00E+10	5.00E+05	2.00E+10	1.00E+10±5.77E+09
168	1.00E+09	2.00E+05	1.00E+08	3.67E+08±3.18E+08
Bacteroides spp.				
0	4.00E+06	1.00E+06	1.90E+08	6.50E+07±6.25E+07
24	2.00E+05	1.00E+06	3.10E+07	1.07E+07±1.01E+07
48	2.40E+07	3.00E+08	3.00E+07	1.18E+08±9.10E+07
72	4.00E+05	2.00E+04	-	2.10E+05±1.55E+05
96	1.00E+07	6.00E+06	1.00E+09	3.39E+08±3.31E+08
168	1.00E+07	1.10E+06	6.00E+07	2.37E+07±1.83E+07
Clostridium spp.				
0	2.00E+03	1.00E+03	1.00E+03	1.33E+03±3.33E+02
24	1.00E+03	2.00E+03	2.00E+03	1.67E+03±3.33E+02
48	-	4.00E+03	-	4.00E+03
72	1.00E+03	1.00E+03	8.00E+04	2.73E+04±2.63E+04
96	1.00E+06	1.00E+08	2.00E+04	3.37E+07±3.32E+07
168	2.00E+05	1.00E+03	2.00E+04	7.37E+04±6.34E+04

Table D10. Counts of viable bacteria per g faeces following intravenous administration of oxytetracycline to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
coliforms					
0	3.00E+05	1.70E+05	4.00E+05	4.00E+04	2.28E+05±7.82E+04
24	4.00E+04	9.00E+03	1.00E+06	1.00E+05	2.87E+05±2.38E+05
48	2.00E+05	1.00E+05	1.00E+06	1.10E+04	3.28E+05±2.27E+05
72	4.00E+05	2.00E+05	1.00E+05	4.00E+05	2.75E+05±7.50E+04
168	1.00E+06	5.00E+06	3.00E+05	1.00E+07	4.08E+06±2.23E+06
streptococci					
0	6.00E+06	2.10E+07	2.00E+07	3.00E+07	1.93E+07±4.96E+06
24	1.00E+07	1.00E+10	3.00E+06	9.00E+06	2.51E+09±2.50E+09
48	1.00E+07	3.00E+08	1.00E+07	4.00E+06	8.10E+07±7.30E+07
72	2.00E+06	2.00E+08	5.00E+05	5.00E+06	5.19E+07±4.94E+07
168	1.00E+07	4.00E+08	6.00E+06	4.00E+07	1.14E+08±9.56E+07
lactobacilli					
0	2.00E+05	1.00E+08	2.00E+05	2.00E+07	3.01E+07±2.38E+07
24	2.00E+07	2.00E+09	3.00E+07	2.00E+07	5.18E+08±4.94E+08
48	-	1.70E+08	-	-	1.70E+08
72	-	7.00E+05	-	4.00E+06	2.35E+06±1.65E+06
168	2.00E+06	-	2.00E+06	-	2.00E+06±0.00E+00
Bacteroides spp.					
0	2.40E+07	1.00E+06	1.00E+07	1.00E+07	1.13E+07±4.75E+06
24	5.00E+09	3.00E+06	6.00E+07	1.00E+06	1.27E+09±1.24E+09
48	-	1.00E+10	-	1.00E+08	5.05E+09±4.95E+09
72	3.10E+07	1.10E+08	1.00E+09	7.00E+07	3.03E+08±2.33E+08
168	3.60E+07	-	1.00E+06	1.00E+10	3.35E+09±3.33E+09
Clostridium spp.					
0	2.00E+05	1.00E+04	6.00E+05	1.00E+04	2.05E+05±1.39E+05
24	1.00E+06	3.00E+03	2.00E+04	3.00E+03	2.57E+05±2.48E+05
48	1.00E+06	8.00E+03	1.00E+06	1.00E+03	5.02E+05±2.87E+05
72	3.00E+05	1.00E+04	1.00E+06	2.00E+04	3.33E+05±2.32E+05
168	-	-	-	1.00E+03	1.00E+03

Table D11. Counts of viable bacteria per g faeces following intravenous administration of oxytetracycline to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
coliforms				
0	4.00E+04	1.00E+05	1.00E+06	3.80E+05±3.10E+05
24	2.00E+06	1.10E+06	2.00E+07	7.70E+06±6.16E+06
48	2.00E+06	1.00E+05	2.00E+06	1.37E+06±6.33E+05
72	5.00E+07	1.00E+06	1.00E+09	3.50E+08±3.25E+08
96	4.00E+06	1.00E+09	3.00E+06	3.36E+08±3.32E+08
168	1.00E+07	1.00E+06	2.00E+06	4.33E+06±2.85E+06
streptococci				
0	1.00E+05	3.00E+06	2.00E+06	1.70E+06±8.50E+05
24	3.00E+04	2.00E+06	3.00E+05	7.77E+05±6.17E+05
48	6.00E+05	1.00E+05	1.00E+07	3.57E+06±3.22E+06
72	4.00E+05	1.30E+05	8.00E+05	4.43E+05±1.95E+05
96	5.00E+05	3.00E+06	2.00E+06	1.83E+06±7.26E+05
168	3.00E+05	1.00E+06	1.00E+07	3.77E+06±3.12E+06
lactobacilli				
0	1.00E+06	2.00E+07	3.00E+06	8.00E+06±6.03E+06
24	1.00E+06	1.00E+06	3.00E+05	7.67E+05±2.33E+05
48	6.00E+05	1.00E+05	1.00E+06	5.67E+05±2.60E+05
72	3.00E+06	3.00E+05	1.00E+09	3.34E+08±3.33E+08
96	1.00E+06	6.00E+06	5.00E+07	1.90E+07±1.56E+07
168	1.00E+07	1.00E+07	4.00E+04	6.68E+06±3.32E+06
Bacteroides spp.				
0	8.00E+04	5.30E+07	1.10E+06	1.81E+07±1.75E+07
24	1.00E+06	1.30E+06	1.00E+07	4.10E+06±2.95E+06
48	1.00E+06	9.00E+04	4.00E+06	1.70E+06±1.18E+06
72	2.00E+07	1.00E+06	2.40E+08	8.70E+07±7.67E+07
96	3.00E+08	2.20E+10	8.00E+07	7.46E+09±7.27E+09
168	2.00E+06	2.00E+06	3.00E+05	1.43E+06±5.67E+05
Clostridium spp.				
0	1.00E+05	-	-	1.00E+05
24	-	-	-	-
48	-	1.00E+04	-	1.00E+04
72	2.00E+05	2.00E+05	-	2.00E+05±0.00E+00
96	NS	NS	NS	NS
168	NS	NS	NS	NS

Table D12. Counts of viable bacteria per g faeces following intravenous administration of oxytetracycline to donkeys

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	2.3
24	0.0	0.0	0.0	0.0	0.0	4.1	0.0	4.1
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	1.4	0.0	0.0	0.0	0.0	1.4
168	0.0	39.2	4.3	0.0	283.6	0.0	0.0	327.1

Table D13. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to horse 1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	1.3	0.0	0.0	0.0	0.0	0.0	1.3
48	17.7	3.2	13.1	0.0	0.0	2.5	0.0	36.5
72	2.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
168	2.8	0.0	6.2	0.0	215.4	0.0	0.0	224.4

Table D14. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to horse 2

Time (h)	L	A	P	IB	B	IV	V	Total
0	4.7	0.0	6.5	0.0	187	0.0	0.0	198.2
24	1.3	2.7	0.0	0.0	16.9	2.0	0.0	22.9
48	57.5	0.0	0.0	0.0	58.8	0.0	0.0	116.3
72	3.3	2.0	0.0	0.0	8.5	0.0	0.0	13.8
96	5.5	48.4	8.0	0.0	183.4	0.0	7.3	252.6
168	5.2	55.6	15.3	0.0	178.6	0.0	0.0	254.7

Table D15. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to horse 3

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	17.9	0.0	0.0	17.9
24	0.0	0.0	0.0	0.0	39.6	0.0	0.0	39.6
48	14.3	13.4	4.4	0.0	166.4	0.0	0.0	198.5
72	3.5	0.0	0.0	0.0	126.1	3.6	0.0	133.2
168	0.0	0.0	0.0	0.0	39.5	0.0	0.0	39.5

Table D16. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony 7

Time (h)	L	A	P	IB	B	IV	V	Total
0	7.1	16.3	0.0	0.0	192.8	0.0	0.0	216.2
24	1.6	0.0	0.0	0.0	227.1	0.0	0.0	228.7
48	9.2	3.0	0.0	0.0	22.5	0.0	0.0	34.7
72	36.5	0.0	0.0	0.0	42.7	0.0	0.0	79.2
168	1.3	0.0	0.0	0.0	20.9	0.0	0.0	22.2

Table D17. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony 8

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	1.9	21.1	0.0	0.0	49.8	0.0	0.0	72.8
72	0.0	0.0	4.3	0.0	9.0	3.6	0.0	16.9
168	2.9	0.0	0.0	0.0	0.0	1.3	3.5	7.7

Table D18. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony 9

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	1.2	0.0	0.0	0.0	0.0	4.0	5.2
24	0.0	0.0	1.5	1.8	0.0	0.0	0.0	3.3
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	1.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1
168	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table D19. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony 10

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	16.5	0.0	147.9	0.0	0.0	164.4
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	6.0	3.0	0.0	9.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
168	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table D20. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to donkey 15

Time (h)	L	A	P	IB	B	IV	V	Total
0	7.6	68.2	7.2	0.0	164.3	0.0	0.0	247.3
24	4.1	16.1	0.0	0.0	85.6	0.0	0.0	105.8
48	1.4	1.7	0.0	0.0	0.0	0.0	0.0	3.1
72	0.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0
96	0.0	0.0	2.9	0.0	0.0	0.0	0.0	2.9
168	0.0	0.0	1.4	0.0	0.0	0.0	0.0	1.4

Table D21. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to donkey 16

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	2.7
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	6.0	0.0	0.0	6.0
96	0.0	0.0	0.0	1.8	0.0	4.4	0.0	6.2
168	0.0	0.0	2.2	3.0	3.0	3.4	0.0	11.6

Table D22. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to donkey 17

	Horses		Ponies		Donkeys	
Time (h)	L	Total	L	Total	L	Total
0	2.3±1.4	66.8±65.7	1.8±1.8	59.8±52.3	2.5±2.5	138.1±71.8
24	0.4±0.4	9.4±6.8	0.4±0.4	67.9±54.4	1.4±1.4	35.3±35.3
48	25.1±17.0	50.9±34.3	6.4±3.3	154.6±76.1	0.5±0.5	4.0±2.6
72	1.8±1.0	5.3±4.3	10.3±8.8	57.6±30.3	0.0±0.0	2.7±1.8
96	1.8±1.8	84.7±84.0	NS	NS	0.0±0.0	3.0±1.8
168	2.7±1.5	268.7±30.5	1.1±0.7	17.4±8.7	0.0±0.0	4.3±3.7

Table D23. SCFA concentrations (mmol/kg) (mean±SEM) in faeces following intravenous administration of oxytetracycline to horses, ponies and donkeys

Time (h)	Horse 1	Horse 2	Horse 3	mean±SEM
0	20.80	20.20	20.00	20.33±0.24
24	15.20	17.40	20.60	17.73±1.57
48	15.80	15.20	20.00	17.00±1.51
72	16.50	17.00	17.60	17.03±0.32
96	14.40	18.90	16.00	16.43±1.32
168	17.60	17.00	18.50	17.70±0.44

Table D24. Faecal dry matter content (%) following intravenous administration of oxytetracycline to horses

Time (h)	Pony 7	Pony 8	Pony 9	Pony 10	mean±SEM
0	17.50	15.60	28.70	12.50	18.58±3.53
24	21.10	13.00	21.10	19.70	18.73±1.94
48	13.00	11.20	20.40	18.50	15.78±2.19
72	13.50	14.30	23.70	20.00	17.88±2.42
168	16.40	17.00	17.40	20.40	17.80±0.89

Table D25. Faecal dry matter content (%) following intravenous administration of oxytetracycline to ponies

Time (h)	Donkey 15	Donkey 16	Donkey 17	mean±SEM
0	23.40	19.50	13.30	18.73±2.94
24	19.00	18.00	13.70	16.90±1.63
48	16.50	10.00	15.30	13.93±2.00
72	13.30	16.50	18.90	16.23±1.62
96	16.70	12.30	19.30	16.10±2.04
168	20.40	18.30	17.00	18.57±0.99

Table D26. Faecal dry matter content (%) following intravenous administration of oxytetracycline to donkeys

Time (h)	I1	I2	II1	II2	III1	III2	III3
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.083	NS	NS	NS	NS	NS	NS	66.72
0.25	26.13	35.13	21.17	24.99	16.35	NS	66.34
0.5	20.08	26.07	13.93	16.50	8.11	34.11	40.19
0.75	11.09	19.08	10.28	14.69	8.42	NS	27.61
1	10.28	15.89	9.37	11.82	7.67	20.07	23.08
1.5	8.19	13.71	6.41	9.78	6.05	NS	13.71
2	7.21	10.42	5.83	10.72	5.90	13.97	10.79
4	4.46	7.40	3.57	6.65	5.74	9.70	6.81
6	NS	NS	NS	NS	6.14	NS	NS
7	NS	NS	NS	NS	NS	7.14	NS
8	2.37	6.58	2.14	3.56	5.45	NS	4.09
12	2.33	4.71	1.62	2.90	5.38	NS	2.75
24	1.31	2.50	0.71	1.86	3.00	2.22	0.89
28	1.09	1.87	0.66	1.45	2.66	NS	NS
31	NS	NS	NS	NS	NS	1.66	NS
32	0.83	1.58	0.59	1.12	2.24	NS	NS
36	0.63	1.46	0.45	1.01	1.58	NS	0.54
48	0.34	0.74	0.31	0.49	0.92	0.72	0.34
52	0.19	0.56	0.22	0.38	0.76	NS	NS
56	0.14	0.52	0.18	0.34	0.73	NS	NS
72	0.00	0.16	0.09	0.13	0.57	0.13	NS
96	0.00	0.00	0.00	0.00	NS	NS	NS
168	0.00	NS	0.00	NS	NS	NS	NS

Table D27. Plasma concentrations (μg/ml) of oxytetracycline following intravenous administration to ponies

Time (h)	I1	I2	II1	II2	III1	III2	III3
0	0.00	0.00	0.00	0.00	0.00	0.00	NS
0.25	0.00	0.00	0.00	0.00	0.00	NS	NS
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	NS	0.23
1	0.00	0.00	0.00	0.00	0.36	NS	0.47
1.5	0.44	0.22	0.00	0.00	0.53	NS	0.44
2	0.46	0.45	0.00	0.00	0.73	NS	2.24
4	1.36	1.37	0.00	0.00	0.62	NS	2.41
6	NS	NS	NS	NS	0.60	NS	NS
7	NS	NS	NS	NS	NS	1.85	NS
8	1.36	1.26	0.17	0.00	0.64	NS	0.35
12	0.78	0.70	0.50	1.52	1.82	NS	0.51
24	0.40	0.00	1.19	0.64	1.02	0.98	2.03
28	0.38	0.00	1.23	0.75	0.44	NS	NS
31	NS	NS	NS	NS	NS	1.12	NS
32	0.29	0.00	1.05	0.00	0.22	NS	NS
36	0.00	0.00	1.23	0.00	0.55	NS	0.23
48	0.00	0.00	0.69	0.00	0.26	0.66	0.44
52	0.00	0.00	0.74	0.00	0.16	NS	NS
56	0.00	0.00	0.45	0.00	0.25	NS	NS
72	0.00	0.00	0.00	0.00	0.00	0.77	NS
96	0.00	0.00	0.00	0.00	NS	NS	NS

Table D28. Caecal liquor concentrations (μg/ml) of oxytetracycline following intravenous administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
24	1.64	3.86	3.01	3.06
48	3.07	1.79	4.89	1.26
72	0.00	0.00	1.48	0.00
96	0.00	0.00	0.00	0.00
168	0.00	0.00	0.00	0.00

Table D29. Faecal concentrations (μg/g) of oxytetracycline following intravenous administration to ponies

Time (h)	I1	I2	II1	II2	III1	III2
coliforms						
0	2.00E+07	3.00E+04	2.00E+06	3.00E+04	1.00E+06	2.00E+09
24	1.00E+07	3.00E+06	1.00E+06	1.00E+06	7.00E+10	1.00E+06
48	1.10E+08	7.00E+05	1.00E+07	6.00E+03	6.00E+08	1.00E+08
72	1.00E+06	4.00E+05	1.10E+05	2.00E+04	NS	1.00E+10
96	9.00E+06	6.00E+05	2.00E+04	5.00E+05	NS	NS
144	NS	NS	NS	NS	NS	4.00E+09
168	1.00E+06	1.00E+06	4.00E+05	2.10E+05	NS	NS
336	NS	NS	NS	NS	9.00E+09	NS
streptococci						
0	1.00E+04	1.00E+05	1.00E+06	2.00E+06	4.00E+08	1.00E+10
24	1.00E+05	7.00E+05	2.00E+08	1.00E+05	8.00E+10	1.00E+08
48	2.00E+06	2.00E+06	2.00E+06	1.00E+06	9.00E+10	3.00E+07
72	1.00E+07	6.00E+05	1.00E+06	2.00E+04	NS	2.00E+08
96	1.00E+05	4.00E+05	3.00E+05	3.00E+05	NS	NS
144	NS	NS	NS	NS	NS	2.00E+09
168	3.00E+05	1.00E+05	2.00E+05	4.00E+04	NS	NS
336	NS	NS	NS	NS	7.00E+10	NS
lactobacilli						
0	1.00E+05	2.00E+06	3.00E+07	1.00E+05	3.00E+06	2.00E+10
24	4.00E+05	6.00E+06	1.00E+07	1.00E+04	5.00E+09	1.00E+06
48	9.00E+07	3.00E+05	4.00E+07	2.00E+05	2.00E+08	-
72	1.00E+07	3.00E+05	1.00E+05	2.00E+05	NS	-
96	2.00E+06	1.00E+06	6.00E+05	1.00E+05	NS	NS
144	NS	NS	NS	NS	NS	2.00E+09
168	1.00E+06	3.00E+05	1.00E+06	2.00E+04	NS	NS
336	NS	NS	NS	NS	-	NS
Bacteroides spp.						
0	7.00E+07	1.00E+10	1.00E+09	1.30E+08	4.00E+05	1.00E+08
24	1.60E+08	1.40E+07	3.00E+08	3.00E+06	-	-
48	3.00E+06	1.30E+07	1.00E+09	2.10E+06	2.00E+05	1.00E+08
72	1.00E+08	1.20E+07	1.00E+08	1.70E+07	NS	1.00E+09
96	2.00E+09	2.20E+07	3.00E+08	2.00E+08	NS	NS
144	NS	NS	NS	NS	NS	2.00E+10
168	5.00E+07	2.20E+08	8.00E+10	4.00E+10	NS	NS
336	NS	NS	NS	NS	-	NS
Clostridium spp.						
0	-	1.00E+06	-	2.00E+04	1.00E+05	1.00E+04
24	-	-	-	2.00E+03	9.00E+03	5.00E+04
48	-	-	-	-	2.00E+04	3.00E+05
72	-	-	-	-	NS	9.00E+04
96	1.00E+04	-	2.00E+03	-	NS	NS
144	NS	NS	NS	NS	NS	2.00E+05
168	1.00E+04	4.00E+05	2.00E+03	2.00E+03	NS	NS
336	NS	NS	NS	NS	2.00E+05	NS

Table D30. Counts of viable bacteria per ml caecal liquor following intravenous administration of oxytetracycline to ponies

Time (h)	I1	I2	II1	II2
0	7.6	7.0	7.5	7.3
0.25	7.0	7.1	7.1	7.4
0.5	7.1	6.9	7.1	7.6
0.75	7.0	7.3	6.9	7.5
1	7.0	6.7	7.1	7.4
1.5	7.4	7.6	7.0	7.3
2	7.6	7.1	7.2	7.2
4	7.6	6.8	7.1	7.3
8	7.0	6.5	8.3	6.9
12	8.0	7.2	8.1	6.9
24	7.0	6.9	7.6	7.6
28	6.8	6.9	7.1	6.8
32	6.8	6.8	7.0	7.2
36	7.0	6.8	6.8	7.1
48	7.1	6.9	7.4	7.4
52	7.6	6.7	7.2	7.2
56	7.1	7.0	7.2	7.0
72	6.8	6.6	7.6	7.4
96	7.0	6.4	7.5	7.6
168	6.6	6.9	7.7	7.7

Table D31. Caecal liquor pH following intravenous administration of oxytetracycline

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.3	28.4	8.2	0.0	11.6	1.0	0.0	49.2
0.25	1.8	33.6	8.8	0.0	13.7	0.0	0.0	56.1
0.5	1.4	34.0	10.6	0.0	11.5	0.0	2.1	58.2
0.75	2.6	34.9	1.6	0.0	11.7	0.0	0.0	48.2
1	0.0	42.6	13.0	0.0	12.4	0.0	0.0	68.0
1.5	3.6	42.0	9.6	0.0	15.8	0.0	0.0	67.4
2	2.3	31.1	6.9	0.0	13.7	0.0	0.0	51.7
4	7.6	18.7	7.3	0.0	12.5	0.0	0.0	38.5
8	11.5	32.1	4.4	0.0	29.5	0.0	0.0	66.0
12	3.7	15.1	1.9	0.0	43.8	0.0	0.0	60.8
24	7.9	26.8	2.3	0.0	13.1	3.3	0.0	45.5
28	10.9	19.3	8.4	0.0	16.0	0.0	0.0	43.7
32	16.4	43.3	6.8	0.0	16.6	0.0	0.0	66.7
36	12.0	40.8	5.4	0.0	14.8	0.0	0.0	61.0
48	9.8	25.6	1.7	0.0	21.5	0.0	0.0	48.8
52	12.2	27.1	2.7	0.0	13.6	0.0	0.0	43.4
56	14.1	31.8	3.1	0.0	35.2	2.6	0.0	72.7
72	12.7	39.4	11.3	0.0	50.7	0.0	0.0	101.4
96	0.0	44.0	10.5	0.0	15.1	0.0	0.0	69.6
168	0.0	39.4	9.1	0.0	10.3	0.0	0.0	58.8

Table D32a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony I1

Time (h)	A	P	B	P+B
0	57.7	16.7	23.6	40.2
0.25	59.9	15.7	24.4	40.1
0.5	58.4	18.2	19.8	38.0
0.75	72.4	3.3	24.3	27.6
1	62.6	19.1	18.2	37.4
1.5	62.3	14.2	23.4	37.7
2	60.2	13.3	26.5	39.8
4	48.6	19.0	32.5	51.4
8	48.6	6.7	44.7	51.4
12	24.8	3.1	72.0	75.2
24	58.9	5.1	28.8	33.8
28	44.2	19.2	36.6	55.8
32	64.9	10.2	24.9	35.1
36	66.9	8.9	24.3	33.1
48	52.5	3.5	44.1	47.5
52	62.4	6.2	31.3	37.6
56	43.7	4.3	48.4	52.7
72	38.9	11.1	50.0	61.1
96	63.2	15.1	21.7	36.8
168	67.0	15.5	17.5	33.0

Table D32b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.6	31.7	7.6	0.0	36.0	0.0	0.0	75.3
0.25	1.3	30.4	4.9	0.0	17.9	0.0	0.0	53.2
0.5	2.1	31.3	6.7	0.0	26.8	0.0	2.5	67.3
0.75	4.0	24.5	4.1	0.0	51.2	0.0	0.0	79.8
1	5.4	29.4	5.9	0.0	54.4	0.0	0.0	89.7
1.5	5.4	55.7	8.5	0.0	79.1	0.0	0.0	143.3
2	4.2	32.2	4.5	0.0	48.1	0.0	0.0	84.8
4	5.0	22.0	5.7	0.0	43.3	0.0	0.0	71.0
8	11.8	13.8	2.5	0.0	121.4	0.0	0.0	137.7
12	9.4	19.2	3.6	0.0	38.2	0.0	0.0	61.0
24	4.4	20.2	5.6	0.0	21.6	0.0	3.3	50.7
28	1.2	18.4	4.0	0.0	18.8	1.9	2.6	45.7
32	3.4	22.9	3.8	0.0	33.5	0.0	0.0	60.2
36	4.0	21.6	3.8	0.0	41.4	0.0	0.0	66.8
48	2.2	23.9	4.3	0.0	23.2	0.0	0.0	51.4
52	3.7	20.5	5.7	0.0	25.2	0.0	0.0	51.4
56	5.4	22.4	5.1	0.0	48.1	0.0	0.0	75.6
72	9.5	24.2	9.4	0.0	11.8	0.0	0.0	45.4
96	0.0	22.0	7.0	0.0	14.0	0.0	2.5	45.5
168	0.0	21.7	8.1	0.0	10.9	0.0	0.0	40.7

Table D33a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony I2

Time (h)	A	P	B	P+B
0	42.1	10.1	47.8	57.9
0.25	57.1	9.2	33.6	42.9
0.5	46.5	10.0	39.8	49.8
0.75	30.7	5.1	64.2	69.3
1	32.8	6.6	60.6	67.2
1.5	38.9	5.9	55.2	61.1
2	38.0	5.3	56.7	62.0
4	31.0	8.0	61.0	69.0
8	10.0	1.8	88.2	90.0
12	31.5	5.9	62.6	68.5
24	39.8	11.0	42.6	53.6
28	40.3	8.8	41.1	49.9
32	38.0	6.3	55.6	62.0
36	32.3	5.7	62.0	67.7
48	46.5	8.4	45.1	53.5
52	39.9	11.1	49.0	60.1
56	29.6	6.7	63.6	70.4
72	53.3	20.7	26.0	46.7
96	48.4	15.4	30.8	46.2
168	53.3	19.9	26.8	46.7

Table D33b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	49.5	12.4	1.0	13.9	0.0	0.0	76.8
0.25	0.0	41.3	11.6	0.0	10.3	1.4	0.0	64.6
0.5	0.0	45.3	11.8	1.0	9.9	0.0	0.0	68.0
0.75	0.0	34.2	6.7	0.0	7.0	0.0	4.5	52.4
1	0.0	43.7	10.1	0.0	8.4	1.3	0.0	63.5
1.5	2.1	46.4	13.0	1.7	7.9	5.1	0.0	74.1
2	0.0	48.2	10.0	0.0	11.5	0.0	0.0	69.7
4	0.0	50.7	12.0	0.0	16.5	1.9	3.9	85.0
8	4.5	40.9	6.2	0.0	25.3	0.0	0.0	72.4
12	3.1	39.7	5.7	0.8	16.7	0.0	0.0	62.9
24	4.5	36.1	7.3	1.4	11.0	2.4	1.6	59.8
28	6.9	24.6	8.1	1.1	10.6	2.4	0.0	46.8
32	16.2	29.1	9.4	1.4	13.2	0.0	0.0	53.1
36	21.0	31.6	11.2	0.0	11.3	0.0	0.0	54.1
48	35.2	19.9	12.1	0.0	17.9	4.2	0.0	54.1
52	35.2	14.5	11.3	0.0	8.9	4.0	4.7	43.4
56	40.2	16.1	10.1	0.0	5.4	3.6	7.3	42.5
72	28.2	24.6	6.0	0.0	9.9	0.0	0.0	40.5
96	0.0	30.3	9.8	1.0	8.1	0.0	0.0	49.2
168	0.0	16.6	9.3	0.0	10.1	1.9	0.0	37.9

Table D34a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony II1

Time (h)	A	P	B	P+B
0	64.5	16.1	18.1	34.2
0.25	63.9	18.0	15.9	33.9
0.5	66.6	17.4	14.6	31.9
0.75	65.3	12.8	13.4	26.1
1	68.8	15.9	13.2	29.1
1.5	62.6	17.5	10.7	28.2
2	69.2	14.3	16.5	30.8
4	59.6	14.1	19.4	33.5
8	56.5	8.6	34.9	43.5
12	63.1	9.1	26.6	35.6
24	60.4	12.2	18.4	30.6
28	52.6	17.3	22.6	40.0
32	54.8	17.7	24.9	42.6
36	58.4	20.7	20.9	41.6
48	36.8	22.4	33.1	55.5
52	33.4	26.0	20.5	46.5
56	37.9	23.8	12.7	36.5
72	60.7	14.8	24.4	39.3
96	61.6	19.9	16.5	36.4
168	43.8	24.5	26.6	51.2

Table D34b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	25.6	7.6	0.0	14.0	0.0	0.0	47.2
0.25	0.0	17.9	4.6	0.0	11.8	0.0	0.0	34.3
0.5	0.0	19.9	6.9	0.0	9.3	0.0	0.0	36.1
0.75	0.0	9.4	3.7	0.0	6.8	0.0	1.0	20.9
1	0.0	17.5	3.0	0.0	7.2	2.6	0.0	30.3
1.5	0.0	20.2	6.9	0.0	10.8	0.0	0.0	37.9
2	0.0	19.9	5.5	0.0	9.7	0.0	0.0	35.1
4	0.0	31.1	7.7	0.0	16.8	0.0	0.0	55.6
8	0.0	31.1	8.9	1.0	22.5	0.0	0.0	63.5
12	0.0	25.8	7.6	0.0	11.5	0.0	0.0	44.9
24	0.1	11.7	6.0	0.0	4.5	0.0	0.0	22.2
28	2.3	16.6	1.8	0.0	7.5	1.0	0.0	26.9
32	2.3	18.8	7.3	0.0	10.1	0.0	0.0	36.2
36	0.0	20.9	10.6	0.0	12.9	0.0	0.0	44.4
48	0.0	20.9	7.1	0.0	6.2	0.0	0.0	34.2
52	0.0	20.5	6.0	0.0	9.2	0.0	0.0	35.7
56	0.0	28.6	10.8	0.0	22.3	0.0	0.0	61.7
72	4.5	14.7	5.9	0.0	5.3	1.2	0.0	27.1
96	2.2	17.4	6.6	0.0	18.9	1.0	0.0	43.9
168	0.0	17.6	5.3	0.0	9.4	0.0	0.0	32.3

Table D35a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony II2

Time (h)	A	P	B	P+B
0	54.2	16.1	29.7	45.8
0.25	52.2	13.4	34.4	47.8
0.5	55.1	19.1	25.8	44.9
0.75	45.0	17.7	32.5	50.2
1	57.8	9.9	23.8	33.7
1.5	53.3	18.2	28.5	46.7
2	56.7	15.7	27.6	43.3
4	55.9	13.8	30.2	44.1
8	49.0	14.0	35.4	49.4
12	57.5	16.9	25.6	42.5
24	52.7	27.0	20.3	47.3
28	61.7	6.7	27.9	34.6
32	51.9	20.2	27.9	48.1
36	47.1	23.9	29.1	52.9
48	61.1	20.8	18.1	38.9
52	57.4	16.8	25.8	42.6
56	46.4	17.5	36.1	53.6
72	54.2	21.8	19.6	41.3
96	39.6	15.0	43.1	58.1
168	54.5	16.4	29.1	45.5

Table D35b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0.25	1.5	28.9	10.4	0.0	6.5	1.4	0.0	47.2
0.5	0.8	29.8	9.9	0.0	5.5	0.0	0.0	45.2
0.75	0.5	33.2	11.8	0.0	6.3	0.0	0.0	51.3
1	0.0	13.7	4.9	0.0	0.8	0.0	0.0	19.4
1.5	1.1	32.0	12.4	0.0	7.1	0.0	0.0	51.5
2	0.0	27.9	6.4	1.0	5.1	1.0	0.0	41.4
4	11.8	15.2	9.8	0.0	4.5	0.0	0.0	29.5
6	3.2	16.8	3.9	0.0	4.8	0.0	0.0	25.5
8	7.3	7.2	21.9	0.0	4.1	0.0	0.0	33.2
12	6.8	13.6	23.6	0.0	3.8	0.0	0.0	41.0
24	5.3	14.6	3.1	0.0	3.0	0.0	0.0	20.7
28	9.5	13.8	20.1	0.0	2.3	0.0	0.0	36.2
32	7.7	23.5	26.7	0.0	4.8	0.0	0.0	55.0
36	8.8	31.6	5.4	0.0	6.8	0.0	0.0	43.8
48	4.6	16.2	2.6	0.0	4.4	0.0	0.0	23.2
52	13.8	26.5	5.7	0.0	2.4	0.0	0.0	34.6
56	12.1	25.1	4.8	0.0	8.1	0.0	0.0	38.0
72	6.3	12.4	3.0	0.0	2.9	0.0	0.0	18.3
144	1.8	32.8	7.1	2.1	2.5	0.0	0.0	44.5

Table D36a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony III1

Time (h)	A	P	B	P+B
0.25	61.2	22.0	13.8	35.8
0.5	65.9	21.9	12.2	34.1
0.75	64.7	23.0	12.3	35.3
1	70.6	25.3	4.1	29.4
1.5	62.1	24.1	13.8	37.9
2	67.4	15.5	12.3	27.8
4	51.5	33.2	15.3	48.5
6	65.9	15.3	18.8	34.1
8	21.7	66.0	12.3	78.3
12	33.2	57.6	9.3	66.8
24	70.5	15.0	14.5	29.5
28	38.1	55.5	6.4	61.9
32	42.7	48.5	8.7	57.3
36	72.1	12.3	15.5	27.9
48	69.8	11.2	19.0	30.2
52	76.6	16.5	6.9	23.4
56	66.1	12.6	21.3	33.9
72	67.8	16.4	15.8	32.2
144	73.7	16.0	5.6	21.6

Table D36b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony III1

Time (h)	L	A	P	IB	B	IV	V	Total
7	13.5	10.1	11.3	0.0	12.0	0.0	0.0	33.4
24	4.4	18.1	4.9	0.0	12.1	0.0	0.0	35.1
31	14.8	24.7	14.3	0.0	10.1	1.8	0.0	50.9
72	20.3	13.4	11.6	0.0	4.1	0.0	0.0	29.1

Table D37a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony III2

Time (h)	A	P	B	P+B
7	30.2	33.8	35.9	69.8
24	51.6	14.0	34.5	48.4
31	48.5	28.1	19.8	47.9
72	46.0	39.9	14.1	54.0

Table D37b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony III2

Time (h)	L	A	P	IB	B	IV	V	Total
0.083	3.9	19.6	11.1	0.0	10.4	0.0	0.0	41.1
0.25	3.9	27.6	16.8	0.0	5.1	0.0	0.0	49.5
0.75	7.5	25.4	21.1	0.0	16.1	2.8	0.0	65.4
1	5.1	26.6	19.3	0.0	11.3	0.0	0.0	57.2
1.5	13.2	29.8	20.0	0.0	10.6	0.0	0.0	60.4
4	29.7	25.8	8.5	0.0	6.2	0.0	0.0	40.5
8	32.6	24.2	7.0	0.0	3.8	0.0	0.0	35.0
12	12.8	17.5	14.2	0.0	12.5	0.0	0.0	44.2
24	3.9	14.1	9.3	0.0	9.7	0.0	0.0	33.1
36	11.0	25.1	5.9	0.0	10.9	0.0	0.0	41.9
48	2.4	14.5	11.1	0.0	9.0	0.0	0.0	34.6

Table D38a. SCFA concentrations (mmol/l) in caecal liquor following intravenous administration of oxytetracycline to pony III3

Time (h)	A	P	B	P+B
0.083	47.7	27.0	25.3	52.3
0.25	55.8	33.9	10.3	44.2
0.75	38.8	32.3	24.6	56.9
1	46.5	33.7	19.8	53.5
1.5	49.3	33.1	17.5	50.7
4	63.7	21.0	15.3	36.3
8	69.1	20.0	10.9	30.9
12	39.6	32.1	28.3	60.4
24	42.6	28.1	29.3	57.4
36	59.9	14.1	26.0	40.1
48	41.9	32.1	26.0	58.1

Table D38b. VFA concentrations (%) in caecal liquor following intravenous administration of oxytetracycline to pony III3

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	14.3	4.2	0.0	18.5
24	0.0	0.0	0.0	0.0	6.7	0.0	0.0	6.7
48	0.0	0.0	8.0	0.0	5.0	0.0	0.0	13.0
72	0.0	0.0	0.0	0.0	8.4	0.0	0.0	8.4
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
168	0.0	0.0	0.0	3.1	15.8	0.0	0.0	18.9

Table D39. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	3.1	15.9	6.4	0.0	67.5	0.0	0.0	92.9
24	2.7	19.5	9.6	0.0	57.4	0.0	0.0	89.2
48	4.5	25.3	13.0	0.0	97.7	0.0	0.0	140.5
72	4.6	22.6	14.6	0.0	112.5	0.0	0.0	154.3
96	3.2	22.6	9.9	0.0	84.3	0.0	0.0	120.0
168	3.1	24.3	5.6	0.0	42.8	0.0	0.0	75.8

Table D40. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	26.9	0.0	0.0	26.9
24	4.2	3.1	0.0	0.0	65.7	0.0	0.0	68.8
48	25.7	13.8	8.9	0.0	102.0	0.0	0.0	124.7
72	0.0	0.0	9.1	0.0	2.3	0.0	0.0	11.4
96	0.0	0.0	5.9	0.0	36.1	0.0	0.0	42.0
168	0.0	0.0	0.0	3.7	15.7	0.0	0.0	19.4

Table D41. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	4.0	17.1	10.2	0.0	117.1	0.0	0.0	144.4
24	9.0	36.7	17.8	0.0	154.0	0.0	0.0	208.5
48	3.6	20.4	9.4	0.0	109.7	0.0	0.0	139.5
72	2.6	19.1	11.2	0.0	94.5	0.0	0.0	124.8
96	4.5	20.4	10.2	0.0	107.7	0.0	0.0	138.3
168	3.9	24.7	6.4	0.0	55.0	0.0	0.0	86.1

Table D42. SCFA concentrations (mmol/kg) in faeces following intravenous administration of oxytetracycline to pony II2

Time (h)	I1	I2	II1	II2
0	23.85	20.20	22.03	20.38
24	24.49	20.31	24.13	23.27
48	22.04	19.17	24.10	21.09
72	18.59	22.13	19.19	21.78
96	18.93	21.08	20.62	21.89
168	19.44	21.16	19.38	20.35

Table D43. Faecal dry matter content (%) following intravenous administration of oxytetracycline to ponies

Time (h)	0	24	48	72	96	168
urea (mmol/l)	1.4	3.0	4.0	3.0	4.1	3.7
sodium (mmol/l)	139	134	132	134	139	137
potassium (mmol/l)	3.6	4.1	3.9	4.3	3.0	2.8
chloride (mmol/l)	102	102	104	104	103	98
calcium (mmol/l)	3.19	3.11	2.84	3.00	2.91	2.98
magnesium (mmol/l)	0.63	0.68	0.62	0.59	0.58	0.63
phosphate (mmol/l)	1.06	1.01	1.20	1.28	1.00	0.76
creatinine (µmol/l)	127	134	144	140	139	154
bilirubin (µmol/l)	9	12	14	10	10	16
SAP (U/l)	345	350	354	333	336	378
AST (U/l)	248	254	254	255	252	273
total protein (g/l)	65	66	66	67	67	71
albumin (g/l)	27	28	28	28	28	30
globulin (g/l)	38	38	38	39	39	41

Table D44. Plasma biochemistry following intravenous administration of oxytetracycline to pony I1

Time (h)	0	24	48	72	96	168
urea (mmol/l)	1.4	2.6	1.7	2.4	3.1	3.7
sodium (mmol/l)	143	141	141	140	141	139
potassium (mmol/l)	4.3	4.8	3.9	4.3	4.1	3.7
chloride (mmol/l)	101	101	107	99	101	98
calcium (mmol/l)	2.66	2.82	2.98	3.04	2.88	2.87
magnesium (mmol/l)	0.62	0.64	0.72	0.66	0.73	0.57
phosphate (mmol/l)	0.95	0.85	1.11	0.81	0.76	0.90
creatinine (µmol/l)	142	150	106	130	148	149
bilirubin (µmol/l)	6	5	8	6	6	5
SAP (U/l)	381	356	457	348	334	354
AST (U/l)	269	278	314	274	279	265
GGT (U/l)	13	17	47	18	13	17
total protein (g/l)	65	69	69	68	66	67
albumin (g/l)	27	28	30	27	27	27
globulin (g/l)	38	41	39	39	39	40

Table D45. Plasma biochemistry following intravenous administration of oxytetracycline to pony I2

Time (h)	0	24	48	72	96	168
urea (mmol/l)	1.8	2.3	3.1	2.7	2.8	2.5
sodium (mmol/l)	133	129	130	126	133	129
potassium (mmol/l)	2.6	2.5	3.6	3.4	3.6	2.4
chloride (mmol/l)	99	102	103	103	104	99
calcium (mmol/l)	2.91	2.94	2.95	3.05	3.13	2.90
magnesium (mmol/l)	0.50	0.60	0.50	0.63	0.61	0.71
phosphate (mmol/l)	0.23	0.25	0.56	1.16	0.94	1.41
creatinine (µmol/l)	89	92	83	86	88	108
bilirubin (µmol/l)	32	26	21	15	11	12
SAP (U/l)	486	550	597	669	629	627
AST (U/l)	427	552	641	666	563	443
total protein (g/l)	65	67	72	75	71	77
albumin (g/l)	31	31	31	32	31	32
globulin (g/l)	34	36	41	43	40	45

Table D46. Plasma biochemistry following intravenous administration of oxytetracycline to pony II1

Time (h)	0	24	48	72	96	168
urea (mmol/l)	0.8	0.9	1.6	2.3	1.8	1.6
sodium (mmol/l)	141	142	140	141	142	141
potassium (mmol/l)	4.2	3.7	4.3	4.7	3.6	3.1
chloride (mmol/l)	105	106	106	102	106	101
calcium (mmol/l)	2.82	2.86	3.12	2.96	2.88	2.79
magnesium (mmol/l)	0.63	0.62	0.72	0.71	0.84	0.73
phosphate (mmol/l)	1.11	0.97	0.99	1.02	0.94	0.93
creatinine (µmol/l)	113	117	102	140	114	132
bilirubin (µmol/l)	8	7	8	6	8	7
SAP (U/l)	480	464	451	325	417	444
AST (U/l)	330	328	314	256	311	327
GGT (U/l)	50	50	47	14	41	48
total protein (g/l)	73	71	71	64	70	74
albumin (g/l)	42	30	31	25	29	32
globulin (g/l)	50	41	40	39	41	42

Table D47. Plasma biochemistry following intravenous administration of oxytetracycline to pony II2

Time (h)	0	24	48	72	144
urea (mmol/l)	3.6	5.8	5.4	6.6	5.7
sodium (mmol/l)	133	130	131	131	125
potassium (mmol/l)	4.8	5.2	3.6	3.4	5.7
chloride (mmol/l)	105	99	102	103	96
calcium (mmol/l)	3.16	2.89	2.68	2.61	2.88
magnesium (mmol/l)	0.74	0.65	0.76	0.56	0.71
phosphate (mmol/l)	1.06	1.26	1.87	1.64	1.31
creatinine (µmol/l)	143	137	153	149	140
bilirubin (µmol/l)	7	10	11	10	10
SAP (U/l)	233	272	273	241	319
AST (U/l)	486	545	548	565	575
ALT (U/l)	26	59	77	58	38
total protein (g/l)	70	74	71	69	70
albumin (g/l)	34	36	36	33	35
globulin (g/l)	36	36	35	36	35

Table D48. Plasma biochemistry following intravenous administration of oxytetracycline to pony III1

Time (h)	0	24	48	72	96	168
WCC (x10^9/l)	9.4	8.8	8.5	9.2	9.1	7.5
RCC (x10^{12}/l)	6.22	6.32	6.29	6.10	6.47	6.75
Hb (g/dl)	10.6	11.0	10.8	10.5	11.3	11.6
Hct (l/l)	0.301	0.298	0.301	0.295	0.313	0.316
MCV (fl)	48.0	47.0	48.0	48.0	48.0	47.0
MCH (pg)	17.0	17.4	17.1	17.2	17.4	17.1
MCHC (g/dl)	35.2	36.9	35.8	35.5	36.1	36.7
PLTS (10^9/l)	-	116	132	125	88	120
MPV (fl)	-	6.1	6.2	6.4	6.4	6.1
PCT (%)	-	0.070	0.081	0.080	0.056	0.073
PDW	-	16.3	16.1	17.1	18.7	19.6
Neu (%)	44.5	54.5	47.0	51.5	43.0	41.9
Lym (%)	54.0	45.0	46.4	43.0	55.5	54.4
Mon (%)	0.5	0.0	3.5	3.5	1.0	2.5
Eos (%)	0.5	0.5	2.0	1.5	0.0	1.1
Bas (%)	0.5	0.0	1.0	0.5	0.5	0.0

Table D49. Haematology parameters following intravenous administration of oxytetracycline to pony I1

Time (h)	0	24	48	72	96	168
WCC (x10^9/l)	7.5	8.3	8.2	8.9	8.7	7.5
RCC (x10^{12}/l)	6.22	6.04	5.80	6.29	6.17	5.95
Hb (g/dl)	10.5	10.4	10.2	10.9	10.7	10.1
Hct (l/l)	0.312	0.303	0.291	0.315	0.309	0.294
MCV (fl)	50.0	50.0	50.0	50.0	50.0	49.0
MCH (pg)	16.8	17.2	17.5	17.3	17.3	16.9
MCHC (g/dl)	33.6	34.3	35.0	34.6	34.6	34.3
PLTS (10^9/l)	56	117	119	126	133	12
MPV (fl)	6.4	6.4	6.3	6.1	5.9	6.2
PCT (%)	0.035	0.074	0.074	0.078	0.078	0.075
PDW	18.7	15.6	15.8	16.3	18.6	16.1
Neu (%)	26.9	41.5	37.4	32.0	37.0	32.0
Lym (%)	70.0	55.0	58.3	65.9	58.5	64.5
Mon (%)	2.0	0.5	3.0	2.0	2.5	1.5
Eos (%)	1.1	3.0	1.0	0.0	1.9	2.0
Bas (%)	0.0	0.0	0.0	0.0	0.0	0.0

Table D50. Haematology parameters following intravenous administration of oxytetracycline to pony I2

Time (h)	0	24	48	72	96	168
WCC (x10^9/l)	6.5	8.2	7.9	9.7	10.8	7.5
RCC (x10^{12}/l)	7.15	7.43	7.03	7.61	6.99	6.99
Hb (g/dl)	11.3	11.5	11.0	11.6	11.0	11.5
Hct (l/l)	0.313	0.327	0.304	0.336	0.310	0.295
MCV (fl)	44.0	44.0	43.0	44.0	44.0	42.0
MCH (pg)	15.8	15.4	15.6	15.5	15.7	16.1
MCHC (g/dl)	36.1	35.1	36.1	35.1	35.4	38.3
PLTS (10^9/l)	71	88	96	107	101	119
MPV (fl)	6.2	6.5	6.6	6.5	7.0	6.8
PCT (%)	0.044	0.057	0.063	0.069	0.070	0.080
PDW	19.3	16.9	18.1	16.9	15.7	17.6
Neu (%)	76.0	76.0	52.0	58.0	71.0	54.5
Lym (%)	22.0	19.0	37.0	32.5	25.0	38.5
Mon (%)	2.0	4.0	10.0	7.0	4.0	5.5
Eos (%)	0.0	0.0	0.5	2.0	0.0	1.1
Bas (%)	0.0	1.0	0.5	0.5	0.0	0.5

Table D51. Haematology parameters following intravenous administration of oxytetracycline to pony II1

Time (h)	0	24	48	72	96	168
WCC (x10^9/l)	5.5	5.7	5.6	5.9	6.5	5.7
RCC (x10^{12}/l)	7.55	7.18	7.18	7.21	7.06	7.30
Hb (g/dl)	11.5	10.8	11.1	10.9	10.8	11.2
Hct (l/l)	0.342	0.324	0.324	0.327	0.318	0.321
MCV (fl)	45.0	45.0	45.0	45.0	45.0	44.0
MCH (pg)	15.2	15.0	15.4	15.1	15.2	15.3
MCHC (g/dl)	33.6	33.3	34.2	33.3	33.9	34.8
PLTS (10^9/l)	110	127	133	120	117	100
MPV (fl)	7.1	7.1	6.7	6.8	6.8	7.0
PCT (%)	0.078	0.090	0.089	0.081	0.079	0.070
PDW	15.4	15.4	14.9	16.1	16.1	15.7
Neu (%)	17.9	23.0	39.5	31.0	39.5	40.0
Lym (%)	69.5	73.0	62.9	65.9	53.5	57.0
Mon (%)	1.0	0.0	6.1	3.0	6.0	3.0
Eos (%)	3.2	1.0	2.0	0.0	0.5	0.0
Bas (%)	8.4	3.0	1.1	0.0	0.5	0.0

Table D52. Haematology parameters following intravenous administration of oxytetracycline to pony II2

Time (h)	0	24	48	72	144
WCC (x10^9/l)	9.6	10.3	10.2	12.5	10.4
RBC (x10^{12}/l)	6.3	6.9	6.9	6.7	7.7
Hb (g/dl)	9.9	10.8	10.7	10.0	12.3
Hct (l/l)	0.292	0.318	0.324	0.319	0.367
MCV (fl)	46.4	46.1	47.0	47.0	48.0
Neu (%)	68.0	64.0	65.0	70.5	63.0
Lym (%)	31.0	30.0	33.0	27.0	34.0
Mon (%)	0.5	4.0	2.0	1.0	2.0
Eos (%)	0.5	1.0	0.0	1.5	1.0
Bas (%)	0.0	1.0	0.0	0.0	0.0

Table D53. Haematology parameters following intravenous administration of oxytetracycline to pony III1

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.15	0.18	0.00	0.00
0.5	0.50	0.41	0.19	0.38
0.75	0.53	0.47	0.49	0.24
1	0.53	0.42	0.29	0.24
1.5	0.41	0.84	0.19	0.19
2	0.55	0.27	0.11	0.11
4	0.10	0.15	0.00	0.00
6	0.00	0.00	0.00	0.00

Table D54. Plasma concentrations (μg/ml) of oxytetracycline following oral administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
0.25	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00
1	13.69	0.00	0.00	0.00
1.5	80.50	48.52	0.00	0.00
2	64.28	61.74	0.00	0.00
4	27.50	21.96	0.00	0.00
6	16.49	11.77	15.69	10.24
8	10.90	9.87	34.11	12.94
12	5.71	7.71	29.75	18.40
24	0.48	0.31	9.71	8.45
28	0.00	0.00	10.87	7.41
32	0.00	0.00	5.50	7.20
48	0.00	0.00	0.00	0.82
52	0.00	0.00	0.00	0.77
56	0.00	0.00	0.00	0.47
72	0.00	0.00	0.00	0.00

Table D55. Caecal concentrations (μg/ml) of oxytetracycline following oral administration to ponies

Time (h)	I1	I2	II1	II2
0	0.00	0.00	0.00	0.00
24	38.01	58.37	45.81	48.27
48	4.01	23.60	8.64	31.55
72	0.00	1.32	0.00	2.03
96	0.00	0.00	0.00	0.00

Table D56. Faecal concentrations (μg/g) of oxytetracycline following oral administration to ponies

Time (h)	I1	I2	I3	II1	II2	II3
coliforms						
0	2.00E+03	3.00E+05	1.40E+05	3.00E+03	1.00E+06	1.00E+04
24	3.40E+11	5.10E+11	1.00E+09	9.00E+10	1.00E+09	1.00E+07
48	4.00E+10	6.00E+05	2.00E+05	1.00E+07	2.00E+07	2.00E+05
72	1.30E+05	4.00E+04	1.00E+05	1.00E+05	1.00E+05	3.00E+03
96	2.00E+09	2.00E+06	7.00E+03	2.00E+04	1.00E+05	1.00E+05
168	1.00E+07	1.00E+09	4.00E+05	3.00E+03	3.00E+04	1.00E+04
336	3.00E+05	1.00E+08	NS	1.00E+06	2.00E+04	NS
streptococci						
0	4.00E+04	4.00E+07	9.00E+05	3.00E+06	5.00E+05	3.00E+04
24	2.10E+11	1.10E+11	1.00E+09	4.80E+11	1.00E+07	2.00E+06
48	1.80E+11	1.00E+08	4.00E+07	4.10E+11	1.00E+06	5.00E+04
72	3.00E+08	5.00E+05	1.60E+06	2.00E+08	1.00E+05	1.00E+04
96	2.00E+05	2.00E+05	8.00E+04	9.00E+05	2.00E+05	1.80E+04
168	4.00E+06	2.00E+05	5.00E+04	3.00E+05	1.00E+05	5.00E+03
336	4.00E+07	8.00E+04	NS	5.00E+05	1.00E+05	NS
lactobacilli						
0	5.00E+07	1.00E+08	NS	1.00E+05	2.00E+09	NS
24	6.00E+10	3.10E+07	NS	2.00E+09	1.00E+06	NS
48	2.00E+10	2.00E+10	NS	8.00E+10	1.00E+08	NS
72	2.00E+10	9.00E+05	NS	1.00E+09	1.50E+06	NS
96	4.00E+06	3.00E+05	NS	3.00E+05	2.00E+05	NS
168	1.00E+08	1.00E+07	NS	3.00E+05	1.00E+06	NS
336	1.00E+08	4.00E+07	NS	2.00E+09	1.00E+06	NS
Bacteroides spp.						
0	8.10E+10	2.30E+12	NS	2.60E+11	9.00E+10	NS
24	1.10E+11	1.00E+11	NS	4.00E+10	1.20E+10	NS
48	4.20E+11	4.00E+10	NS	4.00E+11	1.30E+08	NS
72	1.50E+11	2.10E+10	NS	7.20E+10	4.00E+09	NS
96	3.40E+11	1.20E+10	NS	1.30E+11	1.20E+10	NS
168	2.50E+11	1.00E+11	NS	1.20E+11	6.00E+10	NS
336	2.30E+12	1.40E+11	NS	9.00E+10	6.30E+10	NS
Clostridium spp.						
0	-	-	NS	-	1.00E+04	NS
24	4.00E+06	2.00E+07	NS	1.20E+06	3.00E+04	NS
48	2.30E+06	1.10E+04	NS	1.00E+06	2.10E+04	NS
72	1.00E+06	1.00E+04	NS	1.00E+07	4.00E+03	NS
96	2.00E+04	3.00E+03	NS	3.00E+03	2.00E+03	NS
168	1.00E+05	-	NS	-	-	NS
336	-	-	NS	1.00E+04	-	NS

Table D57. Counts of viable bacteria per ml caecal liquor following oral administration of oxytetracycline to ponies

Time (h)	I1	I2	I3	II1	II2	II3
0	7.0	6.9	7.0	6.9	6.9	6.9
0.25	7.0	6.9	NS	6.8	6.9	NS
0.5	7.1	7.0	NS	6.8	6.9	NS
0.75	7.1	6.9	NS	6.8	6.9	NS
1	7.0	7.0	NS	6.9	7.0	NS
1.5	7.1	7.0	NS	6.8	6.9	NS
2	7.3	7.1	NS	6.8	6.9	NS
4	7.2	7.3	NS	6.8	7.0	NS
8	7.7	7.2	NS	7.0	7.0	NS
12	7.6	7.3	NS	6.9	7.0	NS
24	7.3	7.1	7.1	7.0	7.1	7.0
28	7.2	7.4	NS	7.0	7.2	NS
32	6.6	6.9	NS	7.0	7.1	NS
36	6.6	7.0	NS	7.0	6.9	NS
48	7.3	7.1	7.0	6.7	6.8	7.0
52	6.6	6.8	NS	6.5	6.9	NS
56	6.5	6.5	NS	6.5	6.6	NS
72	7.2	6.8	7.0	6.7	6.9	6.8
96	6.7	6.7	6.7	6.7	6.7	6.8
168	7.0	7.2	6.7	7.0	7.2	6.8
336	6.9	6.9	NS	6.9	6.9	NS

Table D58. Caecal liquor pH following oral administration of oxytetracycline to ponies

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.8	21.0	9.3	1.2	8.5	1.1	0.9	42.0
0.25	0.8	25.5	11.4	0.0	17.9	0.0	0.0	54.8
0.5	0.7	15.8	6.8	0.0	12.2	0.0	0.0	34.8
0.75	1.0	13.5	6.4	0.2	11.1	0.0	0.0	31.2
1	1.0	16.5	7.8	0.0	10.3	0.0	0.0	34.6
1.5	0.7	14.8	5.5	0.2	8.2	0.0	0.0	28.7
2	0.4	11.3	4.2	0.0	8.1	0.0	0.0	23.6
4	14.6	18.2	9.3	0.6	18.0	2.3	2.6	51.0
6	22.6	12.8	16.8	0.0	21.1	0.0	0.0	50.7
8	53.9	20.4	33.5	1.9	23.7	1.8	0.0	81.3
12	50.6	11.6	37.7	0.0	17.6	0.0	0.0	66.9
24	32.9	16.5	2.8	0.0	23.8	0.0	0.0	43.1
28	16.8	9.4	2.5	0.0	20.3	0.0	0.0	32.2
32	43.8	23.5	10.5	0.0	20.6	0.0	3.5	58.1
48	4.1	21.9	18.9	0.0	58.1	0.0	0.0	98.9
52	4.3	26.2	8.0	0.0	29.6	1.3	0.0	65.1
56	5.5	29.7	6.2	0.0	33.8	0.0	0.0	69.7
72	0.8	15.1	5.4	0.0	14.0	0.0	0.0	34.5
96	3.1	29.6	9.9	0.0	9.9	0.4	0.0	49.8
168	0.8	24.8	12.2	0.0	14.6	0.0	0.0	51.6
336	0.4	26.3	13.0	0.0	29.0	0.0	0.0	68.3

Table D59a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony I1

Time (h)	A	P	B	P+B
0	50.0	22.1	20.2	42.4
0.25	46.5	20.8	32.7	53.5
0.5	45.4	19.5	35.1	54.6
0.75	43.3	20.5	35.6	56.1
1	47.7	22.5	29.8	52.3
1.5	51.6	19.2	28.6	47.7
2	47.9	17.8	34.3	52.1
4	35.7	18.2	35.3	53.5
6	25.2	33.1	41.6	74.8
8	25.1	41.2	29.2	70.4
12	17.3	56.4	26.3	82.7
24	38.3	6.5	55.2	61.7
28	29.2	7.8	63.0	70.8
32	40.4	18.1	35.5	53.5
48	22.1	19.1	58.7	77.9
52	40.2	12.3	45.5	57.8
56	42.6	8.9	48.5	57.4
72	43.8	15.7	40.6	56.2
96	59.4	19.9	19.9	39.8
168	48.1	23.6	28.3	51.9
336	38.5	19.0	42.5	61.5

Table D59b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.4	26.3	13.0	0.0	29.0	0.0	0.0	68.3
0.25	0.0	19.2	6.8	0.0	30.4	0.0	0.0	56.4
0.5	1.3	17.9	6.2	0.0	23.2	0.0	0.0	47.3
0.75	0.0	19.0	6.8	0.0	24.4	0.0	0.0	50.2
1	0.0	20.5	7.9	0.0	26.5	0.0	0.0	54.9
1.5	0.9	19.2	6.5	0.0	13.3	0.0	0.0	39.0
2	1.7	16.2	5.6	0.0	13.1	0.0	0.0	34.9
4	20.3	5.3	23.1	0.0	10.6	0.0	0.0	39.0
6	15.3	6.0	15.7	0.0	4.7	0.0	0.0	26.4
8	24.9	5.0	19.1	0.0	6.0	0.0	0.0	30.1
12	12.2	7.0	15.3	0.0	3.4	0.0	0.0	25.7
24	7.0	19.3	3.3	0.0	19.6	0.0	0.0	42.2
28	3.9	18.7	2.3	0.0	18.5	0.5	0.0	40.0
32	3.6	20.7	2.7	0.0	15.6	0.0	0.0	39.0
48	9.7	18.4	3.9	0.0	7.1	0.4	0.0	29.8
52	10.8	20.2	3.6	0.0	8.4	0.0	0.0	32.2
56	18.4	35.3	7.8	0.0	32.6	0.0	0.0	75.7
72	7.7	19.5	11.4	0.9	12.4	1.8	0.0	46.0
96	0.0	26.4	8.8	0.0	30.0	0.0	0.0	65.2
168	2.5	25.7	10.2	0.0	26.9	1.1	0.0	63.9
336	1.3	13.8	4.3	0.0	14.1	0.0	0.0	32.2

Table D60a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony I2

Time (h)	A	P	B	P+B
0	38.5	19.0	42.5	61.5
0.25	34.0	12.1	53.9	66.0
0.5	37.8	13.1	49.0	62.2
0.75	37.8	13.5	48.6	62.2
1	37.3	14.4	48.3	62.7
1.5	49.2	16.7	34.1	50.8
2	46.4	16.0	37.5	53.6
4	13.6	59.2	27.2	86.4
6	22.7	59.5	17.8	77.3
8	16.6	63.5	19.9	83.4
12	27.2	59.5	13.2	72.8
24	45.7	7.8	46.4	54.3
28	46.8	5.8	46.3	52.0
32	53.1	6.9	40.0	46.9
48	61.7	13.1	23.8	36.9
52	62.7	11.2	26.1	37.3
56	46.6	10.3	43.1	53.4
72	42.4	24.8	27.0	51.7
96	40.5	13.5	46.0	59.5
168	40.2	16.0	42.1	58.1
336	42.9	13.4	43.8	57.1

Table D60b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.5	32.0	17.5	0.0	71.2	0.0	0.0	120.7
24	4.5	19.0	6.3	0.0	55.5	0.0	2.5	83.3
48	2.3	20.0	13.9	0.0	43.1	0.0	0.0	77.0
72	2.4	22.6	13.6	0.0	58.1	0.0	0.0	94.3
96	1.7	24.0	16.1	0.0	18.3	9.3	0.0	67.7
168	5.4	46.9	32.3	0.0	29.1	0.0	0.0	108.3

Table D61a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony I3

Time (h)	A	P	B	P+B
0	26.5	14.5	59.0	73.5
24	22.8	7.6	66.6	74.2
48	26.0	18.1	56.0	74.0
72	24.0	14.4	61.6	76.0
96	35.5	23.8	27.0	50.8
168	43.3	29.8	26.9	56.7

Table D61b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony I3

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.4	28.8	8.5	0.5	5.4	0.6	0.0	43.8
0.25	0.0	38.8	12.4	0.3	7.2	0.8	0.0	59.5
0.5	1.2	24.4	8.8	0.9	5.1	1.4	1.1	41.7
0.75	2.4	36.6	10.7	1.1	7.1	1.6	0.0	57.1
1	2.0	30.0	10.4	1.1	5.5	0.0	0.0	47.0
1.5	1.5	38.6	11.5	0.0	9.3	0.0	0.0	59.4
2	0.0	46.9	13.2	0.0	7.6	0.0	0.0	67.7
4	0.8	28.9	8.9	0.0	5.3	0.0	0.0	43.1
6	1.8	19.1	5.9	1.1	5.3	0.0	2.2	33.6
8	3.6	21.0	4.5	0.0	6.2	0.0	0.0	31.7
12	16.4	16.9	4.1	0.0	4.8	0.0	0.0	25.8
24	46.4	9.0	9.3	0.0	4.5	0.0	0.0	22.8
28	37.9	12.9	6.3	0.0	7.7	0.0	0.0	26.9
32	14.4	10.8	6.7	0.0	22.8	0.0	0.0	40.3
48	9.6	20.4	16.0	0.0	7.8	0.0	0.0	44.2
52	13.6	21.4	14.2	0.0	7.1	0.0	0.0	42.7
56	22.4	30.2	18.3	0.0	10.0	0.0	0.0	58.5
72	11.8	21.4	19.2	0.0	8.1	0.0	0.0	48.7
96	5.6	25.0	13.7	0.6	8.1	0.0	0.0	47.4
168	0.4	30.8	14.4	0.0	7.6	0.0	0.0	52.8
336	0.0	34.6	8.6	0.0	11.4	3.5	0.0	58.1

Table D62a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony II1

Time (h)	A	P	B	P+B
0	65.8	19.4	12.3	31.7
0.25	65.2	20.8	12.1	32.9
0.5	58.5	21.1	12.2	33.3
0.75	64.1	18.7	12.4	31.2
1	63.8	22.1	11.7	33.8
1.5	65.0	19.4	15.7	35.0
2	69.3	19.5	11.2	30.7
4	67.1	20.6	12.3	32.9
6	56.8	17.6	15.8	33.3
8	66.2	14.2	19.6	33.8
12	65.5	15.9	18.6	34.5
24	39.5	40.8	19.7	60.5
28	48.0	23.4	28.6	52.0
32	26.8	16.6	56.6	73.2
48	46.2	36.2	17.6	53.8
52	50.1	33.3	16.6	49.9
56	51.6	31.3	17.1	48.4
72	43.9	39.4	16.6	56.1
96	52.7	28.9	17.1	46.0
168	58.3	27.3	14.4	41.7
336	59.6	14.8	19.6	34.4

Table D62b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	34.6	8.6	0.0	11.4	3.5	0.0	58.1
0.25	0.6	29.6	6.7	0.0	8.3	1.1	0.0	45.7
0.5	0.0	33.3	8.0	0.0	11.2	0.0	0.0	52.5
0.75	1.1	32.0	8.1	1.6	8.3	0.0	0.0	50.0
1	0.0	33.4	9.1	0.0	9.4	0.0	0.0	51.9
1.5	0.0	31.2	8.2	0.7	10.4	0.0	0.0	50.5
2	0.0	41.3	10.7	0.0	9.6	0.0	0.0	61.6
4	0.0	34.7	10.7	0.0	12.0	3.1	0.0	60.5
6	0.0	24.5	5.2	0.0	8.8	0.0	0.0	38.5
8	1.1	23.5	5.5	0.0	10.8	0.0	0.0	39.8
12	4.9	24.8	6.2	0.8	8.8	1.9	0.0	42.5
24	7.0	25.8	6.7	0.0	9.8	1.1	0.0	43.4
28	10.3	22.0	4.1	0.0	10.4	0.0	0.0	36.5
32	12.5	19.6	3.8	0.0	4.4	0.9	0.0	28.7
48	21.5	32.8	10.2	0.0	7.9	0.0	0.0	50.9
52	23.0	26.5	8.8	0.0	4.5	1.3	0.0	41.1
56	32.0	33.5	12.1	0.0	11.6	2.2	0.0	59.4
72	7.8	25.8	25.5	0.9	13.1	4.0	0.0	69.3
96	0.8	26.8	10.8	0.0	11.5	0.0	0.0	49.1
168	0.6	36.8	14.2	0.0	17.2	0.0	0.0	68.2
336	0.6	28.0	10.8	0.0	29.2	0.0	0.0	68.0

Table D63a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony II2

Time (h)	A	P	B	P+B
0	59.6	14.8	19.6	34.4
0.25	64.8	14.7	18.2	32.8
0.5	63.4	15.2	21.3	36.6
0.75	64.0	16.2	16.6	32.8
1	64.4	17.5	18.1	35.6
1.5	61.8	16.2	20.6	36.8
2	67.0	17.4	15.6	33.0
4	57.4	17.7	19.8	37.5
6	63.6	13.5	22.9	36.4
8	59.0	13.8	27.1	41.0
12	58.4	14.6	20.7	35.3
24	59.4	15.4	22.6	38.0
28	60.3	11.2	28.5	39.7
32	68.3	13.2	15.3	28.6
48	64.4	20.0	15.5	35.6
52	64.5	21.4	10.9	32.4
56	56.4	20.4	19.5	39.9
72	37.2	36.8	18.9	55.7
96	54.6	22.0	23.4	45.4
168	54.0	20.8	25.2	46.0
336	41.2	15.9	42.9	58.8

Table D63b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.8	35.1	11.8	1.9	14.5	2.9	0.0	66.2
24	3.0	21.4	7.5	0.0	12.6	0.0	0.0	41.5
48	1.3	23.0	12.8	0.0	9.4	0.0	0.0	45.2
72	0.0	36.0	20.8	0.0	10.6	0.0	0.0	67.4
96	0.0	32.2	18.9	0.0	11.5	0.0	0.0	62.6
168	0.0	40.7	14.9	0.0	11.9	0.0	0.0	67.5

Table D64a. SCFA concentrations (mmol/l) in caecal liquor following oral administration of oxytetracycline to pony II3

Time (h)	A	P	B	P+B
0	53.0	17.8	21.9	39.7
24	51.6	18.1	30.4	48.4
48	50.9	28.3	20.8	49.1
72	53.4	30.9	15.7	46.6
96	51.4	30.2	18.4	48.6
168	60.3	22.1	17.6	39.7

Table D64b. VFA concentrations (%) in caecal liquor following oral administration of oxytetracycline to pony II3

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.2	0.0	5.9	0.0	14.1	0.0	0.0	20.0
24	15.4	5.0	6.3	0.0	23.9	0.0	0.0	35.2
48	17.4	2.9	43.7	0.0	18.8	0.0	0.0	65.4
72	1.2	2.9	9.7	0.0	12.0	1.4	0.0	26.0
96	2.2	14.6	7.4	0.0	11.8	4.2	0.0	38.0
168	3.1	26.0	14.5	0.0	12.9	0.0	0.0	53.4
336	1.9	0.0	5.2	0.0	14.4	0.0	0.0	19.6

Table D65. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony I1

Time (h)	L	A	P	IB	B	IV	V	Total
0	1.9	0.0	5.2	0.0	14.4	0.0	0.0	19.6
24	3.7	16.7	5.4	0.0	16.3	0.0	0.0	38.4
48	27.7	13.0	36.4	0.0	4.3	0.0	0.0	53.7
72	58.6	10.7	38.8	0.0	5.7	0.0	0.0	55.2
96	3.2	27.7	14.8	0.0	19.1	0.0	0.0	61.6
168	2.3	0.0	4.2	0.0	33.1	0.0	0.0	37.3
336	2.7	15.3	10.2	0.0	37.6	0.0	0.0	63.1

Table D66. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony I2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	32.2	0.0	0.0	32.2
24	16.9	3.5	35.6	0.0	52.0	0.0	0.0	91.1
48	2.1	0.0	15.8	0.0	60.9	0.0	0.0	76.7
72	3.5	23.4	16.9	0.0	37.9	0.0	0.0	78.2
96	0.0	5.4	8.3	0.0	27.7	0.0	0.0	41.4
168	0.0	20.3	5.2	0.0	38.6	0.0	0.0	64.1

Table D67. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony I3

Time (h)	L	A	P	IB	B	IV	V	Total
0	2.5	5.3	8.1	0.0	40.6	0.0	0.0	54.0
24	55.3	1.3	34.9	0.0	44.5	0.0	0.0	80.7
48	31.1	0.0	24.2	0.0	20.4	0.0	0.0	44.6
72	57.3	5.8	16.5	0.0	13.0	0.0	0.0	35.3
96	1.8	25.7	9.5	0.0	28.7	0.0	0.0	63.9
168	5.0	10.9	8.5	0.0	32.2	0.0	0.0	51.6
336	0.0	0.0	0.0	0.0	27.5	0.0	0.0	27.5

Table D68. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony II1

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	0.0	0.0	0.0	27.5	0.0	0.0	27.5
24	14.3	17.7	7.0	0.0	20.8	0.0	0.0	45.5
48	65.4	11.1	19.3	0.0	29.4	0.0	0.0	59.8
72	63.8	9.8	20.2	0.0	28.2	0.0	0.0	58.2
96	6.6	20.8	9.1	0.0	61.2	0.0	0.0	91.1
168	0.0	0.0	3.3	0.0	87.3	0.0	0.0	90.6
336	4.1	23.0	8.3	0.0	82.9	0.0	0.0	114.2

Table D69. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony II2

Time (h)	L	A	P	IB	B	IV	V	Total
0	0.0	2.0	0.0	0.0	27.9	0.0	0.0	29.9
24	3.6	0.0	30.8	0.0	16.0	0.0	0.0	46.8
48	4.3	0.0	12.6	0.0	70.6	0.0	0.0	83.2
72	3.0	27.0	10.3	0.0	33.1	0.0	0.0	70.4
96	0.0	16.4	10.9	0.0	36.4	0.0	0.0	63.7
168	2.2	14.1	11.3	0.0	43.2	0.0	0.0	68.6

Table D70. SCFA concentrations (mmol/kg) in faeces following oral administration of oxytetracycline to pony II3

Time (h)	I1	I2	I3	II1	II2	II3
0	20.21	21.90	16.55	20.11	19.68	18.33
24	18.99	23.96	20.95	16.93	23.94	18.39
48	17.88	19.40	18.55	17.78	18.61	19.53
72	20.97	17.41	20.71	18.32	17.67	22.87
96	19.68	18.98	20.00	19.68	23.96	19.93
168	18.72	20.94	19.07	18.98	20.54	19.77
336	21.90	24.16	NS	19.68	22.99	NS

Table D71. Faecal dry matter content (%) following oral administration of oxytetracycline to ponies

Time (h)	0	24	48	168	336
urea (mmol/l)	2.4	5.3	4.3	1.7	4.3
sodium (mmol/l)	135	133	134	133	136
potassium (mmol/l)	4.0	4.1	3.9	3.1	4.1
chloride (mmol/l)	95	103	106	99	94
calcium (mmol/l)	2.89	3.0	2.67	3.10	3.05
magnesium (mmol/l)	0.63	0.60	0.66	0.72	0.55
phosphate (mmol/l)	1.11	1.47	1.45	0.95	0.97
creatinine (µmol/l)	133	136	139	139	161
bilirubin (µmol/l)	6	9	9	6	12
SAP (U/l)	276	285	301	289	317
AST (U/l)	289	300	293	306	315
GGT (U/l)	20	19	17	26	18
total protein (g/l)	62	68	70	61	68
albumin (g/l)	25	29	30	27	27
globulin (g/l)	37	39	40	34	41

Table D72. Plasma biochemistry following oral administration of oxytetracycline to pony I1

Time (h)	0	24	48	168	336
urea (mmol/l)	4.3	7.5	5.5	3.7	3.5
sodium (mmol/l)	136	132	134	134	133
potassium (mmol/l)	4.1	4.6	3.5	2.5	3.3
chloride (mmol/l)	94	96	100	98	98
calcium (mmol/l)	3.05	3.04	2.79	2.98	2.98
magnesium (mmol/l)	0.55	0.63	0.56	0.80	0.75
phosphate (mmol/l)	0.97	0.69	1.01	0.63	1.16
creatinine (µmol/l)	161	161	160	133	122
bilirubin (µmol/l)	12	18	19	9	11
SAP (U/l)	317	314	288	288	271
AST (U/l)	315	304	296	302	307
GGT (U/l)	18	29	13	15	17
total protein (g/l)	68	69	66	67	68
albumin (g/l)	27	27	27	27	27
globulin (g/l)	41	42	39	40	41

Table D73. Plasma biochemistry following oral administration of oxytetracycline to pony I2

Time (h)	0	24	48	168
urea (mmol/l)	1.9	4.9	2.3	3.1
sodium (mmol/l)	136	136	138	136
potassium (mmol/l)	4.2	3.9	2.4	2.9
chloride (mmol/l)	98	97	98	101
calcium (mmol/l)	2.78	2.78	2.63	3.07
magnesium (mmol/l)	0.62	0.70	0.66	0.71
phosphate (mmol/l)	1.02	1.11	0.91	0.72
creatinine (µmol/l)	123	103	126	131
bilirubin (µmol/l)	4	16	7	4
SAP (U/l)	276	299	311	280
AST (U/l)	276	335	309	301
GGT (U/l)	26	29	29	26
total protein (g/l)	64	69	72	71
albumin (g/l)	26	29	27	25
globulin (g/l)	38	40	45	46

Table D74. Plasma biochemistry following oral administration of oxytetracycline to pony I3

Time (h)	0	24	48	168	336
urea (mmol/l)	0.6	1.9	1.2	0.5	2.3
sodium (mmol/l)	134	135	134	132	137
potassium (mmol/l)	3.2	3.9	3.1	3.9	3.5
chloride (mmol/l)	100	107	107	100	98
calcium (mmol/l)	2.99	2.92	2.86	3.28	2.96
magnesium (mmol/l)	0.65	0.69	0.64	0.74	0.62
phosphate (mmol/l)	0.97	1.49	2.06	1.02	0.98
creatinine (µmol/l)	116	115	112	116	147
bilirubin (µmol/l)	8	11	6	4	15
SAP (U/l)	321	312	318	441	379
AST (U/l)	353	350	348	357	375
GGT (U/l)	28	24	26	26	21
total protein (g/l)	68	68	71	66	71
albumin (g/l)	28	28	31	27	30
globulin (g/l)	40	40	40	39	41

Table D75. Plasma biochemistry following oral administration of oxytetracycline to pony II1

Time (h)	0	24	48	168	336
urea (mmol/l)	2.3	3.9	3.3	1.8	2.3
sodium (mmol/l)	137	136	134	135	136
potassium (mmol/l)	3.5	3.3	3.7	5.0	3.7
chloride (mmol/l)	98	99	105	100	101
calcium (mmol/l)	2.96	3.18	2.84	2.99	2.90
magnesium (mmol/l)	0.62	0.70	0.63	0.82	0.70
phosphate (mmol/l)	0.98	0.49	0.96	0.94	0.92
creatinine (µmol/l)	147	142	135	109	104
bilirubin (µmol/l)	15	20	11	6	10
SAP (U/l)	379	761	317	312	309
AST (U/l)	375	364	334	359	360
GGT (U/l)	21	31	15	8	21
total protein (g/l)	71	70	65	73	75
albumin (g/l)	30	30	27	31	30
globulin (g/l)	41	40	38	42	45

Table D76. Plasma biochemistry following oral administration of oxytetracycline to pony II2

Time (h)	0	24	48	168
urea (mmol/l)	0.5	4.9	3.4	1.8
sodium (mmol/l)	136	135	140	134
potassium (mmol/l)	4.3	4.4	2.8	3.1
chloride (mmol/l)	101	99	101	100
calcium (mmol/l)	2.88	2.98	2.65	3.04
magnesium (mmol/l)	0.70	0.64	0.70	0.75
phosphate (mmol/l)	1.02	0.93	0.90	0.51
creatinine (µmol/l)	108	113	120	106
bilirubin (µmol/l)	5	7	15	13
SAP (U/l)	293	284	323	287
AST (U/l)	325	291	379	354
GGT (U/l)	24	17	37	27
total protein (g/l)	69	68	78	73
albumin (g/l)	30	26	31	26
globulin (g/l)	39	42	47	47

Table D77. Plasma biochemistry following oral administration of oxytetracycline to pony II3

Time (h)	0	24	48	72	168	336
WCC ($x10^9$/l)	6.9	5.8	5.9	6.4	5.7	7.0
RCC ($x10^{12}$/l)	5.74	5.18	5.57	5.59	5.49	5.52
Hb (g/dl)	9.8	9.2	9.6	9.8	9.4	9.6
Hct (l/l)	0.291	0.259	0.281	0.275	0.273	0.278
MCV (fl)	51	50	50	49	50	50
MCH (pg)	17.0	17.7	17.2	17.5	17.1	17.3
MCHC (g/dl)	33.6	35.5	34.1	35.6	34.4	34.5
PLTS (10^9/l)	80	99	127	110	116	102
MPV (fl)	5.8	6.0	5.5	5.7	5.3	5.8
PCT (%)	0.046	0.059	0.069	0.062	0.061	0.059
PDW	18.9	16.6	18.1	15.7	16.9	17.2
Neu (%)	33.0	46.0	40.4	38.0	38.0	29.0
Lym (%)	63.0	51.0	55.0	60.0	56.9	67.5
Mon (%)	2.5	2.0	1.5	0.0	1.6	2.5
Eos (%)	1.0	1.0	3.0	2.0	3.0	1.0
Bas (%)	0.4	0.0	0.0	0.0	0.5	0.0

Table D78. Haematology parameters following oral administration of oxytetracycline to pony I1

Time (h)	0	24	48	72	168	336
WCC ($x10^9$/l)	7.0	5.6	6.4	6.5	6.3	5.0
RCC ($x10^{12}$/l)	5.52	5.26	5.17	5.61	5.23	5.03
Hb (g/dl)	9.6	9.0	8.9	9.3	8.9	8.3
Hct (l/l)	0.278	0.260	0.257	0.280	0.260	0.250
MCV (fl)	50	49	50	50	50	50
MCH (pg)	17.3	17.1	17.2	16.5	17.0	16.5
MCHC (g/dl)	34.5	34.6	34.6	33.2	34.2	33.2
PLTS (10^9/l)	102	95	105	119	115	124
MPV (fl)	5.8	5.8	5.8	5.7	5.9	6.0
PCT (%)	0.059	0.055	0.060	0.067	0.067	0.074
PDW	17.2	15.5	15.5	15.7	16.9	16.6
Neu (%)	29.0	44.5	37.0	34.0	46.0	40.5
Lym (%)	67.5	54.5	62.0	59.0	53.0	58.9
Mon (%)	2.5	0.5	0.0	4.0	0.5	0.0
Eos (%)	1.0	0.5	1.0	3.0	0.5	0.6
Bas (%)	0.0	0.0	0.0	0.0	0.0	0.0

Table D79. Haematology parameters following oral administration of oxytetracycline to pony I2

Time (h)	0	24	48	72	168
WCC (x10^9/l)	5.9	6.7	6.4	7.2	7.0
RCC (x10^{12}/l)	5.11	5.35	5.70	5.78	5.73
Hb (g/dl)	8.9	9.4	9.9	10.2	10.0
Hct (l/l)	0.264	0.276	0.294	0.297	0.293
MCV (fl)	52.0	52.0	52.0	51.0	51.0
MCH (pg)	17.4	17.5	17.3	17.6	17.4
MCHC (g/dl)	33.7	34.0	33.6	34.3	34.1
PLTS (10^9/l)	113	120	124	118	127
MPV (fl)	5.9	5.7	5.6	6.0	5.6
PCT (%)	0.066	0.068	0.069	0.070	0.071
PDW	16.9	15.7	17.8	16.6	16.0
Neu (%)	36.0	50.0	32.0	30.5	36.0
Lym (%)	61.0	48.0	66.5	66.5	61.5
Mon (%)	2.0	1.5	0.5	2.0	1.0
Eos (%)	1.0	0.5	0.5	1.0	1.5
Bas (%)	0.0	0.0	0.5	0.0	0.0

Table D80. Haematology parameters following oral administration of oxytetracycline to pony I3

Time (h)	0	24	48	72	168	336
WCC (x10^9/l)	6.2	6.1	6.5	6.6	5.8	6.8
RCC (x10^{12}/l)	6.37	6.04	6.04	6.52	6.31	6.52
Hb (g/dl)	10.0	9.4	9.2	10.4	9.8	10.1
Hct (l/l)	0.297	0.278	0.278	0.294	0.287	0.297
MCV (fl)	47	46	46	45	45	46
MCH (pg)	15.6	15.5	15.2	15.9	15.5	15.4
MCHC (g/dl)	33.6	33.8	33.0	35.3	34.1	34.0
PLTS (10^9/l)	90	103	125	105	99	84
MPV (fl)	6.2	6.2	6.2	6.3	6.1	6.6
PCT (%)	0.055	0.063	0.077	0.066	0.060	0.055
PDW	16.1	17.7	17.7	15.8	16.3	15.1
Neu (%)	32.6	46.0	36.0	37.0	40.4	48.5
Lym (%)	61.9	51.0	59.9	58.5	57.5	46.0
Mon (%)	5.0	2.0	3.1	3.0	0.5	1.5
Eos (%)	0.5	1.0	1.0	1.5	1.6	4.0
Bas (%)	0.0	0.0	0.0	0.0	0.0	0.0

Table D81. Haematology parameters following oral administration of oxytetracycline to pony II1

Time (h)	0	24	48	72	168	336
WCC (x10^9/l)	6.8	6.0	6.2	7.0	5.8	6.2
RCC (x10^{12}/l)	6.52	6.28	6.05	6.31	6.87	6.40
Hb (g/dl)	10.1	9.9	9.5	9.7	10.7	9.8
Hct (l/l)	0.297	0.287	0.277	0.287	0.316	0.293
MCV (fl)	46	46	46	45	46	46
MCH (pg)	15.4	15.7	15.7	15.3	15.5	15.3
MCHC (g/dl)	34.0	34.4	34.2	33.7	33.8	33.4
PLTS (10^9/l)	84	88	83	106	79	95
MPV (fl)	6.6	6.8	6.5	6.4	6.7	6.8
PCT (%)	0.055	0.059	0.053	0.067	0.052	0.064
PDW	15.1	14.7	15.3	17.1	16.4	16.1
Neu (%)	48.5	56.5	31.9	34.5	38.3	46.0
Lym (%)	46.0	39.5	63.1	60.5	59.6	53.0
Mon (%)	1.5	3.5	1.9	2.0	2.1	0.0
Eos (%)	4.0	0.5	3.1	2.0	0.0	1.0
Bas (%)	0.0	0.0	0.0	1.0	0.0	0.0

Table D82. Haematology parameters following oral administration of oxytetracycline to pony II2

Time (h)	0	24	48	72	168
WCC (x10^9/l)	6.3	5.9	6.6	7.2	6.6
RCC (x10^{12}/l)	6.84	6.90	7.14	7.50	7.14
Hb (g/dl)	10.7	10.9	11.2	11.9	11.6
Hct (l/l)	0.323	0.322	0.334	0.349	0.336
MCV (fl)	47.0	47.0	47.0	47.0	47.0
MCH (pg)	15.6	15.7	15.6	15.8	16.2
MCHC (g/dl)	33.1	33.8	33.5	34.0	34.5
PLTS (10^9/l)	103	99	103	118	96
MPV (fl)	6.4	6.5	6.3	6.4	6.4
PCT (%)	0.065	0.064	0.064	0.075	0.061
PDW	17.1	16.9	17.4	15.6	17.1
Neu (%)	39.5	41.0	34.5	46.0	56.5
Lym (%)	56.0	54.5	61.5	52.0	40.5
Mon (%)	2.5	2.0	2.0	2.0	1.5
Eos (%)	1.5	2.0	2.0	0.0	1.5
Bas (%)	0.5	0.5	0.0	0.0	0.0

Table D83. Haematology parameters following oral administration of oxytetracycline to pony II3

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.00	0.00±0.00
1	0.00	0.59	0.37	0.30	0.32±0.12
5	3.02	6.03	3.42	4.74	4.30±0.68
10	7.54	9.36	8.69	8.57	8.54±0.38
20	17.51	17.31	18.2	16.80	17.46±0.29
40	34.61	42.94	28.65	30.87	34.27±3.14
80	54.93	81.05	54.19	50.37	60.14±7.04

Table D84a. Concentrations (µg/ml) of oxytetracycline in caecal liquor following incubation *in vitro* for 3 h

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
0.25	0.00	0.00	0.00	0.00	0.00±0.00
1	0.32	0.45	1.31	0.00	0.52±0.28
5	4.49	4.43	5.76	4.32	4.75±0.34
10	8.79	11.29	12.17	6.69	9.74±1.24
20	15.73	7.66	23.45	15.73	15.64±3.22
40	29.17	29.63	35.73	26.59	30.28±1.94
80	46.02	48.88	65.87	63.46	56.06±5.03

Table D84b. Concentrations (µg/ml) of oxytetracycline in caecal liquor following incubation *in vitro* for 24 h

Time (h)	1	2	3	4	mean±SEM
coliforms					
0	1.00E+04	1.00E+04	2.00E+06	4.00E+05	6.05E+05±4.74E+05
3	1.80E+04	3.00E+04	1.00E+05	5.00E+05	1.62E+05±1.14E+05
24	1.00E+05	1.00E+05	1.00E+04	2.00E+03	5.30E+04±2.72E+04
streptococci					
0	3.00E+05	7.00E+04	1.00E+05	1.00E+05	1.43E+05±5.30E+04
3	4.00E+05	2.00E+05	2.00E+05	8.00E+03	2.02E+05±8.00E+04
24	3.00E+06	2.00E+06	2.00E+04	3.00E+04	1.26E+06±7.43E+05
lactobacilli					
0	1.00E+06	4.00E+07	4.00E+04	2.00E+05	1.03E+07±9.90E+06
3	1.00E+06	1.00E+08	3.00E+05	3.00E+05	2.54E+07±2.49E+07
24	1.00E+06	1.00E+06	1.00E+05	1.00E+04	5.28E+05±2.73E+05
Bacteroides spp.					
0	7.10E+10	1.90E+11	2.50E+07	1.40E+08	6.53E+10±4.48E+10
3	5.00E+10	1.40E+11	7.20E+07	1.30E+08	4.76E+10±3.30E+10
24	6.10E+10	1.20E+11	8.50E+07	4.90E+07	4.53E+10±2.87E+10
Clostridium spp.					
0	3.00E+03	-	1.00E+03	1.00E+03	1.67E+03±6.67E+02
3	2.00E+03	1.00E+06	-	-	5.01E+05±4.99E+05
24	1.00E+06	-	-	-	1.00E+06

Table D85a. Counts of viable bacteria per ml caecal liquor following *in vitro* incubation of caecal liquor

Time (h)	1	2	3	4	mean±SEM
coliforms					
0	1.00E+04	1.00E+04	2.00E+06	4.00E+05	6.05E+05±4.74E+05
3	4.00E+04	6.00E+03	4.00E+03	3.00E+04	2.00E+04±8.91E+03
24	7.00E+10	1.00E+05	7.00E+04	1.00E+03	1.75E+10±1.75E+10
streptococci					
0	3.00E+05	7.00E+04	1.00E+05	1.00E+05	1.43E+05±5.30E+04
3	2.00E+05	2.00E+04	1.00E+05	6.00E+04	9.50E+04±3.86E+04
24	1.00E+06	2.00E+06	1.00E+06	6.00E+03	1.00E+06±4.07E+05
lactobacilli					
0	1.00E+06	4.00E+07	4.00E+04	2.00E+05	1.03E+07±9.90E+06
3	2.00E+06	1.00E+07	5.00E+05	1.00E+05	3.15E+06±2.32E+06
24	1.00E+07	1.00E+06	4.00E+05	1.00E+06	3.10E+06±2.30E+06
Bacteroides spp.					
0	7.10E+10	1.90E+11	2.50E+07	1.40E+08	6.53E+10±4.48E+10
3	4.10E+10	4.10E+10	1.00E+07	1.20E+08	2.05E+10±1.18E+10
24	1.20E+10	5.00E+10	6.00E+06	6.40E+07	1.55E+10±1.18E+10
Clostridium spp.					
0	3.00E+03	-	1.00E+03	1.00E+03	1.67E+03±6.67E+02
3	1.00E+03	-	1.00E+03	-	1.00E+03
24	4.00E+06	-	2.00E+03	-	2.00E+06±2.00E+06

Table D85b. Counts of viable bacteria per ml caecal liquor following *in vitro* incubation with 80 µg/ml oxytetracycline

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.7	37.1	14.2	0.0	13.5	0.3	0.0	65.1
0.25	0.1	39.3	12.2	0.0	15.2	0.0	0.0	66.7
1	0.6	35.8	13.2	0.0	16.1	0.3	0.0	65.4
5	0.0	37.1	12.1	0.0	21.4	0.0	0.0	70.6
10	0.4	34.0	12.4	0.0	19.4	0.0	0.7	66.5
20	0.0	39.7	10.9	0.0	21.4	0.0	0.0	72.0
40	0.5	29.6	9.8	0.0	15.5	0.0	0.0	54.9
80	0.0	34.7	13.0	0.0	21.2	0.0	0.0	68.9

Table D86a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 3 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.3	46.8	21.0	0.5	11.2	0.0	0.0	79.5
0.25	0.0	38.7	16.8	0.0	9.1	0.6	0.0	65.2
1	0.0	42.7	17.7	0.0	9.3	0.0	0.0	69.7
5	0.0	41.6	15.8	0.2	9.9	0.0	0.0	67.5
10	0.0	44.1	16.0	0.0	9.3	0.0	0.0	69.4
20	0.0	38.3	14.2	0.6	8.9	0.0	0.0	62.0
40	0.0	35.4	12.4	0.0	18.5	0.0	0.0	66.3
80	0.2	35.3	15.0	0.0	21.2	0.0	0.0	71.5

Table D86b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 24 h (replicate 1)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	40.3	12.1	0.0	9.7	0.0	0.0	62.1
0.25	2.3	32.5	9.9	2.2	9.5	2.3	0.0	56.4
1	0.5	37.6	10.5	1.1	9.8	0.0	2.3	61.3
5	2.2	36.9	12.8	1.6	11.4	3.3	2.4	68.4
10	1.3	34.2	9.1	0.0	9.1	0.0	0.0	52.4
20	3.2	34.3	10.3	2.9	10.7	3.6	5.4	67.2
40	0.5	35.4	11.5	0.0	10.9	1.6	0.0	59.4
80	2.8	33.8	10.2	0.0	15.5	0.0	0.0	59.5

Table D87a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 3 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	39.5	11.7	0.8	8.6	0.0	0.0	60.6
0.25	1.2	47.8	15.0	1.4	10.9	2.4	2.6	80.1
1	0.0	47.6	15.4	0.0	10.2	0.0	0.0	73.2
5	0.6	40.7	13.4	1.0	10.7	2.6	0.0	68.4
10	0.0	38.3	10.6	0.0	7.8	0.0	0.0	56.7
20	1.3	39.8	11.0	1.8	9.4	3.6	0.0	65.6
40	0.0	37.8	10.3	0.8	7.6	0.0	0.0	56.5
80	1.8	42.2	15.0	2.7	9.2	3.2	0.0	72.3

Table D87b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 24 h (replicate 2)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.6	28.3	12.2	1.5	7.1	0.0	2.3	51.4
0.25	2.2	18.7	10.2	1.9	7.1	2.6	0.0	40.5
1	0.3	31.0	10.8	0.0	6.0	0.5	0.0	48.3
5	2.3	28.1	11.3	2.3	8.6	3.0	2.4	55.7
10	0.5	29.4	11.4	0.4	7.5	1.2	0.0	49.9
20	2.2	31.9	12.9	2.0	9.6	2.4	3.0	61.8
40	0.5	30.4	10.9	0.0	7.5	1.0	0.0	49.8
80	2.3	26.3	10.5	2.3	14.5	4.3	2.3	60.2

Table D88a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 3 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.3	31.0	12.1	0.8	6.0	1.3	0.0	51.2
0.25	3.1	27.4	11.9	3.1	7.1	4.2	3.7	57.4
1	0.3	26.9	12.8	0.0	5.3	0.0	0.0	45.0
5	1.8	31.4	13.2	1.4	7.2	2.2	0.0	55.4
10	0.4	28.7	11.4	0.0	5.7	1.6	0.0	47.4
20	1.8	30.1	13.0	1.7	8.2	1.9	1.3	56.2
40	0.9	30.5	10.5	0.0	6.1	2.4	0.0	49.5
80	3.8	31.2	16.5	2.0	11.7	4.2	4.3	69.9

Table D88b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 24 h (replicate 3)

Conc (µg/ml)	L	A	P	IB	B	IV	V	Total
0	1.3	38.3	11.9	1.0	7.4	1.3	0.0	59.9
0.25	0.0	26.1	12.6	0.0	7.8	1.2	0.0	47.7
1	0.9	33.3	10.5	1.0	7.5	1.4	0.0	53.7
5	0.0	41.6	14.7	0.0	11.1	0.0	0.0	67.4
10	1.3	38.1	12.8	1.1	10.9	2.3	0.0	65.2
20	0.0	41.8	14.1	0.0	12.2	0.0	0.0	68.1
40	3.5	51.6	17.4	0.0	12.9	0.0	0.0	81.9
80	0.0	42.7	12.5	0.0	14.0	0.0	0.0	69.2

Table D89a. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 3 h (replicate 4)

Conc. (µg/ml)	L	A	P	IB	B	IV	V	Total
0	0.0	42.8	11.1	0.0	8.8	0.0	0.0	62.7
0.25	1.0	40.7	11.3	1.6	8.2	2.5	2.0	66.3
1	0.3	37.8	10.5	0.1	6.3	0.8	0.8	56.3
5	0.7	47.7	13.6	0.6	8.3	1.9	0.0	72.1
10	0.0	51.8	14.5	0.0	11.8	0.0	0.0	78.1
20	1.3	52.2	13.1	1.1	11.2	3.1	1.9	82.6
40	1.4	46.9	14.1	1.5	10.5	2.3	0.0	75.3
80	0.0	44.7	15.0	0.0	13.8	0.0	0.0	73.5

Table D89b. SCFA concentrations (mmol/l) in caecal liquor following *in vitro* incubation with oxytetracycline for 24 h (replicate 4)

	3 h		24 h	
Conc (µg/ml)	L	Total	L	Total
0	0.9±0.4	59.6±2.9	0.4±0.3	63.5±5.9
0.25	1.2±0.6	52.9±5.7	1.3±0.6	70.9±5.7
1	0.6±0.1	57.1±3.8	0.2±0.1	61.1±6.5
5	1.1±0.6	65.5±3.4	0.8±0.4	70.0±6.9
10	0.9±0.2	58.5±4.3	0.1±0.1	62.9±6.8
20	1.4±0.8	67.3±2.1	1.1±0.4	66.6±5.7
40	1.3±0.8	61.5±7.1	0.6±0.3	61.8±5.6
80	1.3±0.8	64.5±2.6	1.5±0.9	71.8±0.8

Table D90. SCFA concentrations (mmol/l) (mean±SEM) in caecal liquor following *in vitro* incubation with oxytetracycline

Conc. (µg/ml)	1	2	3	4	mean±SEM
0	0.00	0.00	0.00	0.00	0.00±0.00
1	1.05	1.09	0.95	0.63	0.93±0.10
2	2.07	2.17	2.62	1.82	2.17±0.17
5	4.91	4.60	3.33	5.04	4.47±0.39
10	8.64	11.05	8.01	8.97	9.17±0.66

Table D91. Concentrations (µg/ml) (mean±SEM) of oxytetracycline following incubation *in vitro* at pH 1.9 for 1 h