Title: Examination of the pharmacokinetic/pharmacodynamic (PK/PD) relationships of orally administered antimicrobials and their correlation with the therapy of various bacterial and mycoplasmal infections in pigs

Thesis submitted in accordance with the requirements of the Royal College of Veterinary Surgeons for the Diploma of Fellowship by: -

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Abstract

Examination of the pharmacokinetic/pharmacodynamic (PK/PD) relationships of orally administered antimicrobials and their correlation with the therapy of various bacterial and mycoplasmal infections in pigs

David G S Burch

The use of antimicrobial drugs in pigs to control bacterial and mycoplasmal infections is extremely common. In some countries, like the United Kingdom, it is estimated that over 50% of veterinary antimicrobial use is in pigs. It was the purpose of this thesis to help veterinarians to better understand their use of antibiotics by examining basic pharmacokinetic and pharmacodynamic (PK/PD) relationships in comparison with clinical response, to enable them to improve their clinical success.

Regarding common porcine respiratory infections, there was a clear PK/PD relationship between plasma concentration of the antibiotic using the area under the curve (AUC) and the minimal inhibitory concentration (MIC) of *Mycoplasma hyopneumoniae*. With the bactericidal drug, enrofloxacin, a fluoroquinolone, when a predictable AUC/MIC relationship of >100 could be achieved, the clinical response was good. With the bacteriostatic drugs, the relationship was less clear until the minimum bactericidal concentration (MBC) was used. When it came to the respiratory bacteria, in particular *Actinobacillus pleuropneumoniae*, with those drugs that did not concentrate greatly in lung tissue, such as the fluoroquinolones and the tetracyclines, there was a predictable AUC/MIC relationship, regarding efficacy. However, with the antibiotics such as tilmicosin, tiamulin and tulathromycin, which concentrate in lung and leucocyte cells to a large extent, no plasma relationship could be established. This has led to the conclusion that leucocyte concentrations were more significant.

Regarding enteric infections, a model for estimating small intestinal concentrations of an antimicrobial compound was developed. Data on the colonic or faecal concentrations of antimicrobials were often reported and from the model, approximately 29% of the colonic concentration was used to determine the ileal and 25% the jejunal concentrations and also the AUC. The antimicrobial concentrations in the jejunum corresponded well to *Escherichia coli* susceptibility patterns comparing survey and clinical isolate MICs. New intracellular MIC data have been recently developed for *Lawsonia intracellularis*. There was a good correlation between ileal contents concentrations and AUCs derived from the model and the intracellular MICs and susceptibility patterns that had emerged. *Brachyspira hyodysenteriae* and *B. pilosicoli* were also examined in relation to colonic contents concentration (CCC) and AUC. The method of MIC determination, whether in broth or agar had an impact, with agar MICs being considered close to MBCs. The CCC and AUC/MIC relationship correlated well with the clinical outcome, especially when potential protein-binding figures were used, derived from plasma. In spite of deficiencies in the available published data and the variability in MIC determination, PK/PD principles could generally be applied to both respiratory and enteric infections in the pig.

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List of abbreviations

AGP – Antimicrobial growth promoter
AUC - Area under the curve
CBP – Clinical break point
CCC – Colon contents concentration
CLSI – Clinical and Laboratory Standards Institute
Cmax – Maximum concentration
CTC – Chlortetracycline
ECOV – Epidemiological cut-off value
EDC – Extra-cellular drug concentration
eMIC – Extracellular minimum inhibitory concentration
EP – Enzootic pneumonia
FCE – Feed conversion efficiency
FP – Feed premix
h - Hour
HPLC – High pressure liquid chromatography
ICC – Ileal contents concentration
iMIC – Intracellular minimum inhibitory concentration
I/N – Intra-nasal
Inj. – Injectable product
I/T – Intra-tracheal
LC – Liquid chromatography
MBC – Minimum bactericidal concentration
MDAs – Maternally derived antibodies
MFS – Medicated feedingstuff
MIC – Minimum inhibitory concentration
MMC – Minimum mycoplasmacidal concentration
MPC – Mutant prevention concentration
MS – Mass spectroscopy
MSW – Mutant selection window
NCCLS – National Committee for Clinical Laboratory Standards
OD – Oral doser
PAE – Post-antibiotic effect
PBP – Penicillin-binding protein
PCV2 – Porcine circovirus type 2
PD – Pharmacodynamics
PK – Pharmacokinetics
PMWS – Post-weaning multisystemic wasting syndrome
PPB – Plasma-protein binding
PRDC – Porcine respiratory disease complex
PRRSV – Porcine reproductive and respiratory syndrome virus
WS – Water soluble

Common calculations

A number of PK/PD calculations are commonly used in this thesis, which relate to PK/PD relationships and are summarised here for convenience: -

1. Maximum concentration (Cmax) divided by minimum inhibitory concentration (MIC). For bactericidal antimicrobial drugs such as aminoglycosides and fluoroquinolones, the ratio that gives a bactericidal and possible eliminatory effect is usually ≥10

Cmax (µg/ml) / MIC (µg/ml) = ≥10: 1

2. Area under the curve 24h (AUC_{24h}) divided by MIC. This is also used for bactericidal antibiotics and where the MIC is approximately equivalent to the minimum bactericidal concentration (MBC) and can be applied to aminoglycosides, fluoroquinolones, penicillins and trimethoprim/sulphonamide combinations. A bactericidal effect can be normally achieved at ≥100h.

AUC_{24h} (µg.h/ml) / MIC (µg/ml) = ≥100 h

This can apply to primarily bacteriostatic drugs when the MBC is used instead of the MIC, e.g. for the tetracyclines, macrolides, lincosamides and pleuromutilins.

AUC_{24h} (µg.h/ml) / MBC (µg/ml) = ≥100 h

There is an inhibitory effect when the AUC_{24h} / MIC is ≥ 24h

AUC_{24h} (µg.h/ml) / MIC (µg/ml) = ≥24 h

The inhibitory effect increases to a bactericidal and possibly eliminatory effect as the free-drug concentration, primarily in the plasma but also in extracellular fluids and gut contents, divided by the MIC or MBC, depending on the type of drug, approaches the ≥100h.

3. Time above the MIC (T>MIC) is also important as many antibiotics require time to destroy bacteria. The time is assessed out of a 24 h period and is expressed as a percentage.

T >MIC = e.g. 12h / 24h = 50%

Some antibiotics like the penicillins can exert a bactericidal effect against Gram +ve organisms when the MIC is exceeded for ≤50% of the time. The majority of antibiotics require a longer period and against Gram –ve bacteria, penicillins usually require 100% for a bactericidal effect.

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David Miller also played an important part of my life as mentor in the pharmaceutical industry over the last 30 years and Professor Stanley Done, who gave me the belief in myself and encouragement to attempt this project, while we worked together on the Pig Journal.

Lastly but not least, to Professor Peter Lees, who as an inspired teacher, helped us as students at the RVC to understand pharmacology and to examine the way medicines worked. I must thank him for his continued encouragement, support and supervision in the production of this thesis.

Declaration
The work in this thesis has not already been submitted or accepted in substance for any other degree or comparable qualification

David G. S. Burch **Date 8th February 2012**

Chapter 1. Antimicrobial use in pigs and their pharmacokinetic / pharmacodynamic (PK/PD) relationships

1.1 Introduction

Antimicrobials have been widely used in swine production over several decades and it was reported in 1999 (*Vivash-Jones, 2000) to be worth an estimated $1.7 billion dollars or 34% of the global animal health antimicrobial market, closely followed by poultry (33%) and cattle (26%). It is therefore important for veterinarians to understand how they work and what effect they are likely to have on the bacteria that they are infecting their patients. It is the study of the drug's pharmacokinetics, absorption, distribution, metabolism and excretion in the animal and its pharmacodynamic activity on the bacterium, susceptibility, mode of action, whether bactericidal or bacteriostatic and rate of kill that can influence their choice and resulting treatment success.

It is the purpose of this thesis to draw together data on the pharmacokinetics and pharmacodynamics of various antibiotics that are commonly used in pigs and to compare these parameters, with special regard to treatment outcomes following their use for both respiratory and enteric diseases.

1.2 Bacterial infections in swine, disease patterns and infection interactions

Enteric infections

The common enteric bacterial infections are summarised in Table 1.1.

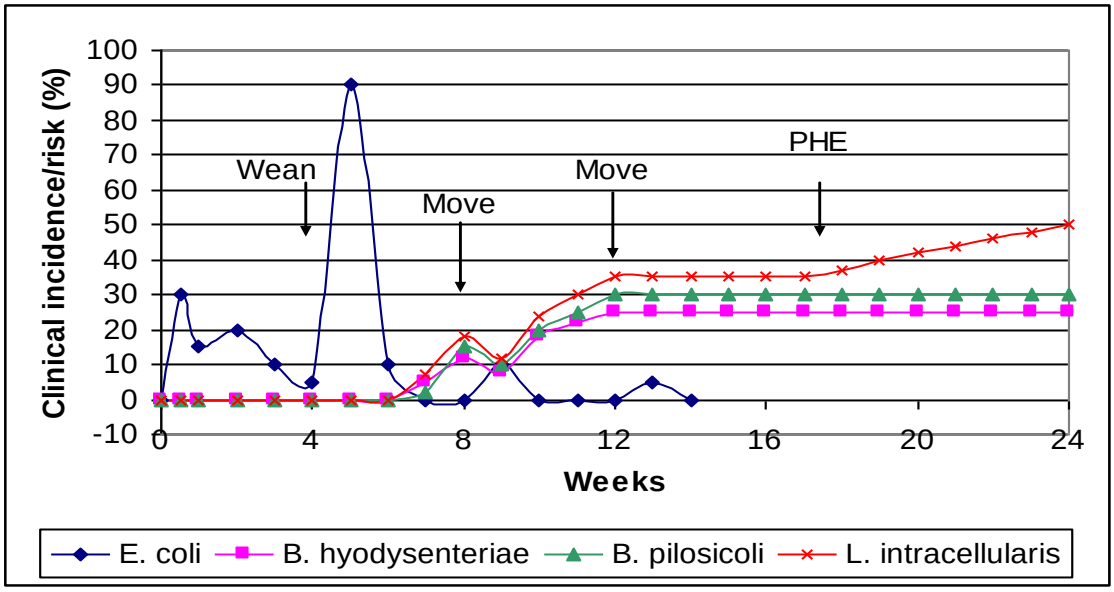
Table 1.1. Common enteric bacterial infections and diseases in the pig (Burch *et al*, 2008)

Bacterium	Disease	Age
Escherichia coli	Neonatal scours Piglet scours Post-weaning diarrhoea Mastitis, metritis, agalactia (MMA) syndrome	1-3 days 7-14 days 5-14 days after weaning Sows, post parturient
Clostridium perfringens	Type C – necrotic enteritis Type A - diarrhoea	1-7 days 10-21 days, weaned pigs
Salmonella enterica spp.	*S.* Typhimurium – occasional diarrhoea, septicaemia, death. *S.* Derby – occasional diarrhoea *S.* Choleraesuis – septicaemia, diarrhoea, death (not UK)	Grower pigs – from weaning onwards – 40% of UK herd serologically +ve. Grower pigs Finishing pigs 12-16 weeks
Lawsonia intracellularis	Porcine proliferative enteropathy (ileitis)	Grower pigs

Bacterium	Disease	Age
	Regional/necrotic ileitis Porcine haemorrhagic enteropathy	Grower pigs Finishing pigs and young adults 16-40 weeks
Brachyspira hyodysenteriae	Swine dysentery	Growers and finishers, 6-26 weeks All ages in primary breakdown
Brachyspira pilosicoli	Intestinal spirochaetosis 'colitis'	Grower pigs

Escherichia coli infections are common and occur from the first days of life. Normally in the pig, *E. coli* cause gut infections but neonatal infections can lead to septicaemia and cause high mortality (see Figure 1.1).

Figure 1.1. Common enteric disease patterns in pigs (Burch *et al*, 2008)



Clostridium perfringens type C is also associated with per-acute haemorrhagic and necrotic enteritis in young piglets, which can be fatal.

Many strains of salmonella have a low pathogenicity in pigs and are more of a concern for zoonotic transmission. However, *S. enterica* Typhimurium can be invasive and can cause septicaemia and death (see Figure 1.3).

Lawsonia intracellularis is a relatively ubiquitous organism on pig farms. A survey in the UK showed that 95% of farms were infected (*Mortimer *et al*, 2000). It is also commonly associated with diarrhoea in growing pigs in Denmark and the UK and primarily affects the ileum although the organism can be found in caecal and colonic epithelial cells. The chronic form in grower pigs is associated with soft faeces and unevenness in growth. The acute haemorrhagic form is usually seen in pigs greater than

60kg bodyweight and in young gilts and boars and results in sporadic mortality but in very valuable animals.

Brachyspira species are also quite common, and the more pathogenic form *B. hyodysenteriae*, the cause of swine dysentery, remains a recurring, severe problem in many countries, including UK, Belgium and Spain, although it is low in the US, possibly associated with the almost universal use of slatted floors. *Brachyspira hyodysenteriae* can cause severe diarrhoea, commonly with mucus and blood and leads to rapid wasting, dehydration and death. *Brachyspira pilosicoli* is quite widespread as a low grade cause of mucoid diarrhoea, either alone or in mixed infections.

Respiratory infections

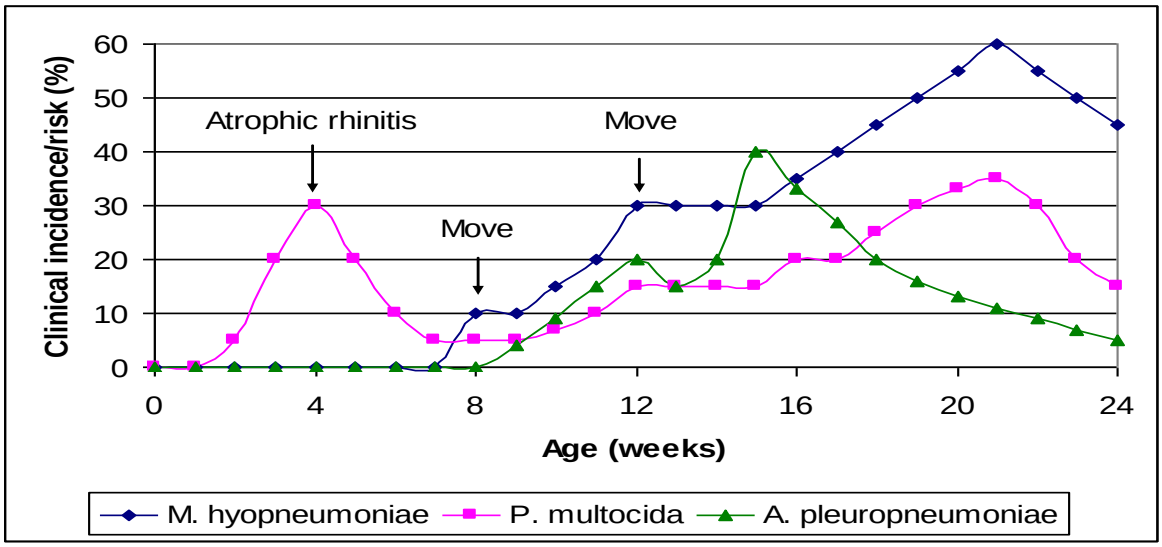
Many porcine bacteria can be found in the respiratory tract, but some can also be found systemically and cause meningitis, arthritis, pleurisy, pericarditis and peritonitis (see Tables 1.2 and 1.3).

Table 1.2. Common respiratory bacteria and diseases in the pig

Organism	Disease	Age
Pasteurella multocida (D) *Bordetella bronchiseptica*	Progressive atrophic rhinitis	1-8 weeks Nasal distortion lasts for life
Mycoplasma hyopneumoniae	Enzootic pneumonia	Grower and finisher pig
Pasteurella multocida	Mycoplasma-induced respiratory disease (MIRD)	Grower and finisher – secondary invader
Actinobacillus pleuropneumoniae	Pleuropneumonia	Grower and finisher – MDA can last for 10 weeks

Atrophic rhinitis is caused by a mixed infection of *B. bronchiseptica* and *P. multocida* and usually starts in young pigs from 7-10 days of age (see Figure 1.2.).

Figure 1.2. Respiratory disease patterns in the pig (Burch *et al*, 2008)



Mycoplasma hyopneumoniae, the cause of enzootic pneumonia, is another endemic disease throughout the world, with most herds being infected. On its own, it normally causes a relatively mild disease, depressing growth rate and feed conversion efficiency (FCE) but in naive herds, in primary herd breakdown, the infection can be quite severe, causing death. The damage it does to the cilia, lining the respiratory tract and the immuno-suppressive effect it has in the lung, permits a number of bacteria, especially *P. multocida*, to colonise the lung and cause broncho-pneumonia, which in severe cases can be fatal. Mycoplasma vaccines have become the main method for successful control with some countries vaccinating over 70% of the national growing herd.

Actinobacillus pleuropneumoniae can cause primary acute necrotising pneumonia on its own or in combination with *M. hyopneumoniae*. Some serotypes given in artificial infection studies can cause death within 24 hours, due to the toxic shock produced by its toxins. Fortunately, it is not as widely spread as enzootic pneumonia, but usually is a more severe infection. Antibacterial treatment is the same as for *P. multocida* and a number of bacterin vaccines have been produced but do not offer complete protection against all serotypes. Sub-unit vaccines are more effective across the 15 serotypes.

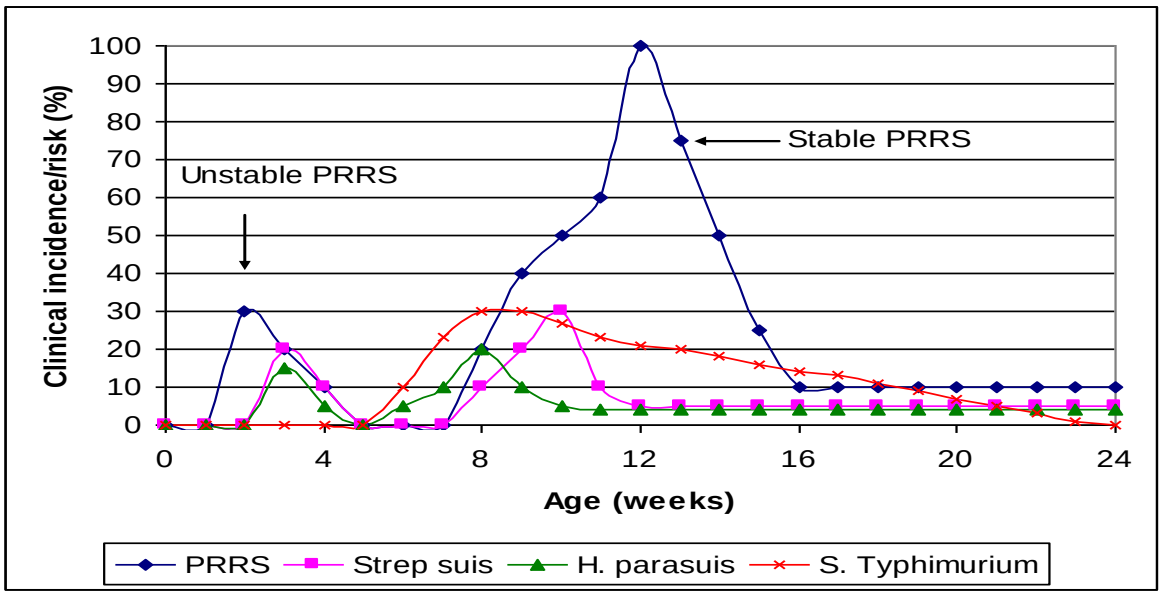
Bacteria that cause septicaemia in pigs

Table 1.3. Common bacteria that may cause septicaemia in the pig

Organism	Disease	Age
Streptococcus suis	Meningitis, arthritis	2-10 weeks
Haemophilus parasuis	Glässer's disease (arthritis, polyserositis, pericarditis, peritonitis)	2-10 weeks
Mycoplasma hyosynoviae	Mycoplasmal arthritis	16 weeks plus
Erysipelas rhusiopathiae	Erysipelas (dermatitis, arthritis, endocarditis)	Growers, finishers and sows/boars

With the introduction of porcine reproductive and respiratory syndrome virus (PRRSV) new patterns of infection appeared. The virus destroys lung macrophages, which then allows a number of minor infections to invade the lung and cause pneumonia and also penetrate into the blood stream and cause septicaemia and the associated conditions (see Figure 1.3).

Figure 1.3. Septicaemic disease patterns (Burch et al, 2008)



Streptococcus suis is quite widespread in many herds but does not always cause clinical problems. Commonly, *S. suis* type 2 is associated with meningitis in weaner and grower pigs, which are overcrowded and have poor ventilation. Following active PRRSV infection, *S. suis* can cause coughing in young piglets from 16 days of age but occasionally even outbreaks of meningitis in finishers. Similarly, *H. parasuis* also causes clinical signs in weaners and growers, especially arthritis (Glässer's disease), when maternally derived antibodies (MDAs) fade. Both infections are thought to be transmitted from sows to piglets in the first week of life. Stabilisation of the immunity of PRRSV infection in the sow herd by vaccination is important, to prevent the early pre-weaning infections.

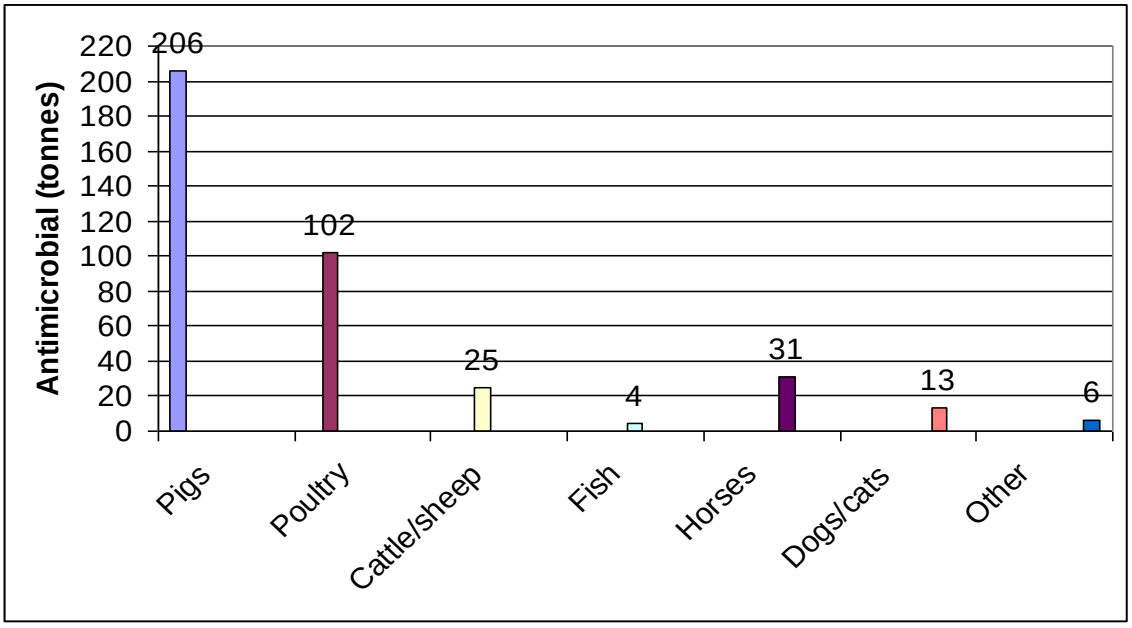
1.3 Antimicrobial use in pigs

Precise figures regarding tonnages of active ingredient and actual use in pigs are rarely published but national bodies are starting to collate total antimicrobial usage in animals and some can break them down by family of antimicrobial (Anon., 2008a; Anon., 2008b) and by species of animal (Denmark) in which they are used (Anon., 2005). Unfortunately, the Danish market is not completely representative, as antimicrobial usage is relatively low. However, tetracyclines dominate in most pig markets, followed by the macrolide/lincosamide/pleuromutilin groups of compounds, but the penicillin, trimethoprim/sulphonamide combination and aminoglycoside use is also important.

Fluoroquinolone and cephalosporin use remains comparatively small in animal use, especially in comparison with use in humans (Anon., 2007a).

In the UK, the Veterinary Medicines directorate (VMD) (Anon., 2008a) published their figures for antimicrobial use in 2007 by family of antimicrobial, route of administration and where possible by species. Of the 387 tonnes of antimicrobial used in animals 45% were tetracyclines, 19% trimethoprim/sulphonamide combinations, 19% beta-lactam antibiotics (penicillins) and 9% macrolides. Eighty-nine per cent was administered orally, 53% in medicated feed premixes, which are primarily used in pigs and poultry. Approximately, 10% are given by injection. It was estimated by the author (DB) that approximately 53% (206 tonnes) of therapeutic antimicrobials are used in pigs; making them the major animal species in the UK for antimicrobial consumption (see Figure 1.4).

Figure 1.4. Antimicrobial consumption by species in the UK (Estimation DB)



The cost/effective disease control plus relative ease of administration, especially via the feed or drinking water, has probably allowed substantial quantities of antimicrobial agents to be used in pigs. There have been concerns expressed by the European Food safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (Anon., 2011a) that high levels of antimicrobial resistance has been found in both *Salmonella* spp and *Campylobacter* spp, potentially zoonotic infections transmissible to man. The EFSA Panel on Biological Hazards (BIOHAZ) have also issued a report (Anon., 2011b) concerning the public health risks of bacterial strains producing extended-spectrum beta-lactamases (ESBLs) and or AmpC beta-lactamases in food and food producing animals. They consider an effective control measure would be to stop all uses of cephalosporins or restrict their use in veterinary medicine and, as co-resistance is also an important potential issue, it is of high priority to decrease the total antimicrobial use in animal production in the EU. However, from the author's (DB) perspective, it is also the education of the veterinarian and the farmer to look for

improved biosecurity, hygiene and management to control infections, which will make a difference and improving the understanding of how antibiotics work will also play a key role. The understanding of their pharmacokinetic and pharmacodynamic relationships in relation to clinical effect will improve their selection and the way they are used.

In general, antimicrobials are considered as **therapeutic** agents to treat infections. In many cases, they have been built into the production system for either **metaphylactic** use, in the case a group is likely to be infected e.g. at weaning, or showing low levels or early signs of disease, then the group is given a high treatment level to try to eliminate the infection and prevent the disease developing in the animals for the rest of the production period. **Prophylaxis** is usually when low levels of the antimicrobial are included for a more prolonged period in feed. **Growth promotion** is often a grey area between low level prevention and sub-disease controlling levels. Particularly in the US, antimicrobial feed premixes may be approved for treatment, prevention and growth promotion. In Europe, since the banning of antimicrobial growth promoters (AGPs) in 2006, antimicrobial premixes for medicated feedingstuffs (MFS) are supplied under a veterinary controlled MFS prescription for prevention or treatment purposes, although the mills generally purchase and include the premixes in the feed. In some countries, like Denmark, they are supplied via the pharmacist.

Feed traditionally has lent itself well to the prophylactic/metaphylactic medication of pigs, as it could easily be built into a strategic medication programme for disease control, without having to physically handle the animal.

Therapeutic antibiotics may be given in feed but are increasingly being given as **soluble formulations** in the drinking water to groups or houses of animals. This method of administration is becoming more popular with the development of more reliable dosing/water-proportioner machines such as the 'Dosatron®'. **Injectables** and **piglet dosers** are also used, but require individual handling of the animal. When they are small, piglets are easy to handle, so metaphylactic injection programmes of antimicrobials are quite common to fight off piglet infections, such as neonatal scours, caused by *E. coli* and early *S. suis* or *H. parasuis* infections. Older pigs are injected later when they are ill but usually for therapy only. A sick pig is easier to inject in a pen or group. As they recover, it is more difficult and can be positively dangerous in large unrestricted sows in yards, hence the development and popularity of **long-acting formulations**, so that they do not have to be injected repeatedly.

The major antimicrobial families and actives used in pig production are described (see Table 1.4.). Not all are approved for use in the UK.

Table 1.4. Major antimicrobial families, active substances, formulations (Inj – injection; OD – oral doser; WS – water soluble/solution; FP – feed premix) and their use in pigs

Family/antimicrobial	Formulations	Use/indication
Tetracyclines:		*M. hyopneumoniae*
Oxytetracycline	Inj, WS, FP	*P. multocida*
Chlortetracycline	WS, FP	*A. pleuropneumoniae*

Family/antimicrobial	Formulations	Use/indication
Tetracycline Doxycycline	WS Inj, WS, FP	*H. parasuis* *L. intracellularis* *E. coli* (resistance) *Salmonella spp* (resistance)
Trimethoprim / sulphonamide:	Inj, OD, WS, FP,	*P. multocida* *B. bronchiseptica* *A. pleuropneumoniae* *S. suis* *S. hyicus* *H. parasuis* *L. intracellularis* *E. coli* *Salmonella spp*
Penicillins: Procaine penicillin (Penicillin G) Phenoxymethyl penicillin (Penicillin V)	 Inj, FP WS, FP	*S. suis* *P. multocida* *H. parasuis* *A. pleuropneumoniae* *A. pyogenes* *C. perfringens* *E. rhusiopathiae*
Synthetic penicillins: Amoxycillin Ampicillin Plus clavulanic acid (beta-lactamase inhibitor)	 Inj, WS, FP Inj, WS Inj, WS (few countries)	*S. suis* *P. multocida* *H. parasuis* *A. pleuropneumoniae* *A. pyogenes* *C. perfringens* *E. rhusiopathiae* *E. coli* *Salmonella spp*
Cephalosporins: Cephalexin (1st Gen) Ceftiofur (3rd Gen) Cefquinome (4th Gen)	 Inj Inj Inj	*S. suis* *P. multocida* *H. parasuis* *A. pleuropneumoniae* *A. pyogenes* *C. perfringens* *E. rhusiopathiae* *E. coli* *Salmonella* spp
Fluoroquinolones: Enrofloxacin Danofloxacin Marbofloxacin	 Inj, OD Inj Inj	*M. hyopneumoniae* *P. multocida* *A. pleuropneumoniae* *H. parasuis* *E. coli* *Salmonella* spp
Thiamphenicols:		*P. multocida*

Family/antimicrobial	Formulations	Use/indication
Thiamphenicol Florfenicol	Inj Inj, WS, FP	*A. pleuropneumoniae* *H. parasuis* *S. suis* *B. bronchiseptica*
Aminoglycosides: Streptomycin Neomycin Apramycin Gentamicin Amikacin *Aminocyclitol:* Spectinomycin	 Inj Inj, OD, WS, FP OD, WSP, FP Inj Inj Inj, OD, WS, FP (+ lincomycin)	*E. coli* *Salmonella* spp *Staphylococcus* spp
Polymixin: Colistin	 OD, WS, FP	*E. coli* *Salmonella spp*
Macrolides: Tylosin Tylvalosin Spiramycin Tilmicosin *Triamilide:* Tulathromycin	 Inj, WS, FP WS, FP FP WS, FP Inj	*M. hyopneumoniae* *L. intracellularis* *B. hyodysenteriae* (resistance) *B. pilosicoli* (resistance) *Plus A. pleuropneumoniae* *H. parasuis* *P. multocida* *S. suis* (resistance)
Lincosamides: Lincomycin	 Inj, WS, FP	*M. hyopneumoniae* *M. hyosynoviae* *L. intracellularis* *B. hyodysenteriae* *B. pilosicoli*
Pleuromutilins: Valnemulin Tiamulin	 FP Inj, WS, FP	*M. hyopneumoniae* *M. hyosynoviae* *L. intracellularis* *B. hyodysenteriae* *B. pilosicoli* *Plus A. pleuropneumoniae*
Miscellaneous: *Growth promoters (not EU):* Avoparcin Virginiamycin Bacitracin Flavophospholipol Avilamycin Carbadox Olaquindox	 FP FP FP FP FP FP FP	 *B. hyodysenteriae* *E. coli*

Family/antimicrobial	Formulations	Use/indication
Anticoccidials:		
Toltrazuril	OD, WS	*Isospora suis*
Salinomycin	FP	
Monensin	FP	

1.4 Improving antimicrobial understanding and use with PK/PD relationship analysis

The pharmacokinetics of antimicrobials are sometimes poorly understood by veterinarians in practice and this is one of the main reasons that the author's interest developed in this area.

The **pharmacokinetics of a drug** can be described as the absorption into, distribution within and elimination from the body (Lees *et al*, 2006). It describes and predicts the drug concentration time profiles in plasma and other biological fluids including the putative biophase. **Pharmacodynamics** encompasses the spectrum of antimicrobial activity and quantitative measures of the potency, efficacy, sensitivity and kill rate of bacteria (Lees *et al*, 2006). The main use of therapeutic antimicrobial drugs is to select an effective drug, in a suitable formulation, at a suitable dose rate to ensure efficacy against a susceptible organism. The drug has to be present at the site of infection, for a sufficient time, in sufficient concentration, to achieve optimal bacteriological and clinical cure. Insufficient destruction of an organism may lead to the development of resistance either by selection of mutants, or transmission of resistance genes, most commonly by plasmid transmission, especially by *Enterobacteriaciae*.

There are a number of key aspects to pig medicine, which is important and specific. The bulk of antimicrobials used in pigs are via the feed, possibly over 80% in some countries. Dose intake is directly linked to feed intake and inclusion level. One of the common problems veterinarians encounter regarding efficacy is potential under-dosing. If the animal is sick or with a high temperature, it will often stop eating. Age of pig is also important. Most dose rates are based upon a 20 kg pig eating 1 kg of feed/day or 5% of bodyweight. Finishing pigs were often given restricted feed to control fat deposition, in male castrates especially, and there can be a halving of relative feed intake to 2.5% from 80kgs and above. This is less common now in the UK with entire males and improved pig genetics. Lactating sows are usually fed to about 2.5% and dry sows can be fed at a rate as low as 1% of bodyweight. To achieve a target dose of chlortetracycline to treat a uterine infection in dry sows, five times the normal inclusion is therefore required. Most in-feed administration gives relatively lower, flatter plasma levels than by injection, especially with products that are metabolised in the liver, such as the macrolides and pleuromutilins, following the slower gastric emptying and passage down the gut. This also reduces their overall bioavailability as judged by plasma concentration. Products excreted via the kidney such as trimethoprim/ sulphonamides and penicillins are not normally so affected. It must also be remembered that some products given orally are not absorbed from the gut to any great extent, such as the aminoglycosides, aminocyclitols and polymixins, so it is of little use to give them orally for systemic or respiratory infections. Soluble products, given via the drinking water or

in liquid feed, tend to pass through the stomach more quickly and are therefore more quickly absorbed and higher therapeutic concentrations can generally be achieved to treat lung infections e.g. tiamulin.

Table 1.5. Major antimicrobials used in pigs and their dosage rates (mg/kg bodyweight) by formulation

Antimicrobial	Injection	In water	In feed
Tetracyclines:			
Oxytetracycline	10 (LA forms 20-30)	10-30	20
Chlortetracycline		20	10-20
Tetracycline		20-40	
Doxycycline	4-6	5	5
Trimethoprim / sulphonamide:	15	30	15
Penicillins:			
Pen G	10 (LA forms 20)	-	-
Pen V	-	10	10
Synthetic penicillins:	7 (LA forms15)	20	15-20
Amoxycillin	7.5	-	-
Ampicillin	+1.75	-	-
Plus clavulanic acid			
Cephalosporins:			
Cephalexin	7	-	-
Ceftiofur	3 (LA forms 5)	-	-
Cefquinome	1-2	-	-
Fluoroquinolones:			
Enrofloxacin	2.5	-	-
Danofloxacin	1.25	-	-
Marbofloxacin	2	-	-
Thiamphenicols:			
Thiamphenicol	10-30	-	10
Florfenicol	15	15	15
Aminoglycosides:			
Streptomycin	25	-	-
Neomycin	- (Not approved)	11	11
Apramycin	-	7.5-12.5	4-8
Gentamicin	- (Not approved)		
Amikacin	- (Not approved)		
Aminocyclitol:			
Spectinomycin	- (Not approved)	10-50	2.2 (+ lincomycin)
Polymixin:			
Colistin	-	50,000iu	50,000iu
Macrolides:			
Tylosin	2-10	25	3-6 (treat) 1.2-2.4 (prevent)

Antimicrobial	Injection	In water	In feed
Tylvalosin	-	-	2.5-5
Tilmicosin	-	-	8-16
Triamilide:			
Tulathromycin	2.5	-	-
Lincosamides:			
Lincomycin	10	4.5	5.5-11 (treat) 2.2 (prevent) 1.1-2.2 (+ spectinomycin)
Pleuromutilins:			
Valnemulin	-	-	3.75-10 (treat) 1.0-1.5 (prevent)
Tiamulin	10-15	8.8-20	5-10 (treat) 2 (prevent)

1.5 Basic pharmacokinetic parameters used for PK/PD integration and modelling

A number of basic pharmacokinetic parameters have emerged, which can be used both for PK/PD integration and possibly PK/PD modelling. They were originally derived from the use of bactericidal antimicrobials, such as the aminoglycosides and fluoroquinolones, to improve the efficacy of treatment of human patients, not only to give clinical cure but to also eliminate the infectious agent, from patients, who may be immuno-compromised and could not fight the infection themselves (Schentag, 2000).

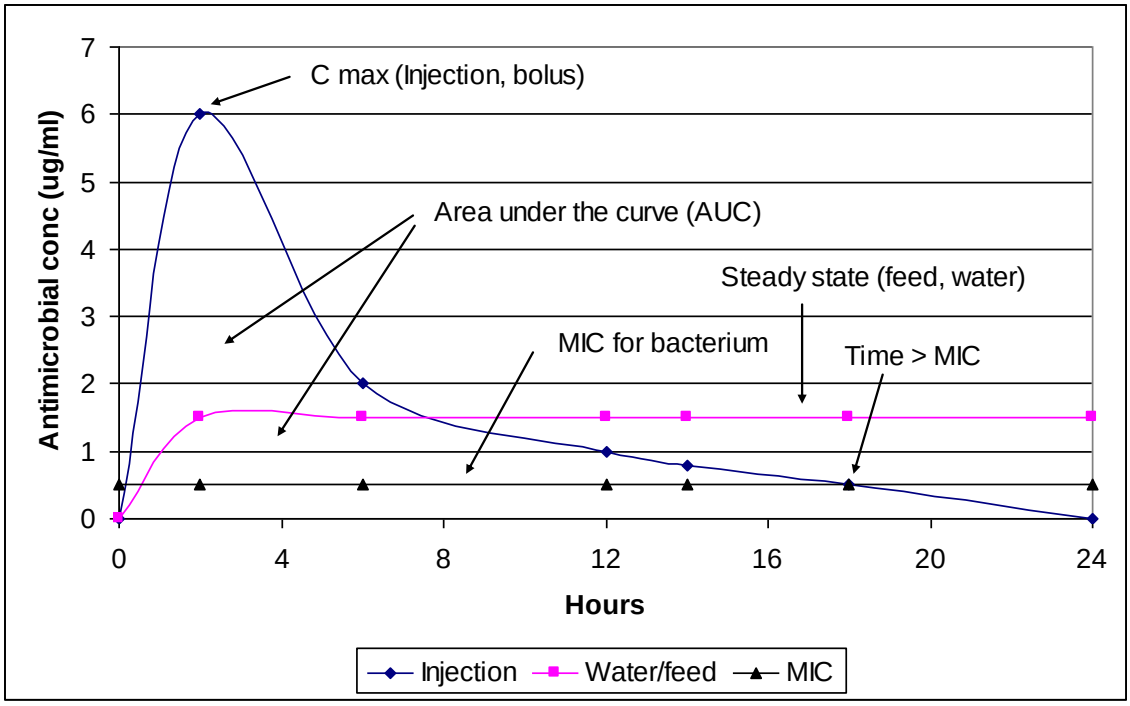
The **Cmax** or maximum concentration of an antimicrobial achieved following administration, usually by injection or a bolus dose (see Figure 1.5). This figure can be used for bactericidal antimicrobials such as aminoglycosides and fluoroquinolones. The Cmax figure when divided by the minimum inhibitory concentration (MIC) of the antibiotic against the organism used, needed to be a value of **10-12** to get a bactericidal and eliminatory effect. The MIC is the concentration of drug that inhibits the growth of the bacterium when grown in vitro and hopefully, this mirrors the effect of the drug in vivo. For example, a product which gave a plasma Cmax of 6µg/ml divided by an MIC of 0.5µg/ml gives a **Cmax/MIC value** of 12. The concentration in plasma should be of **free drug**, not bound to plasma-proteins for precision but this data is not always available.

This concept works well for those bactericidal antimicrobials where the minimum bactericidal concentration / minimum inhibitory concentration **(MBC/MIC) ratio** is close to **one.** Unfortunately, many of the antimicrobials used in pig medicine are bacteriostatic and the MBC/MIC ratio is greater than one and often only inhibitory effects are achieved but the immune system is usually intact.

The **area under the curve over 24 hours (h) (AUC_{24h})** is another useful parameter for PK/PD integration and comprises a concentration and a time or exposure function.

Again, this is widely used for bactericidal antimicrobials and the AUC_{24h} divided by the MIC gives a value of **100-125 h** to be successful therapeutically (Schentag, 2000; Toutain, 2003; Lees *et al*, 2006: Lees *et al*, 2008). The AUC/MIC ratio can also be used for penicillins and trimethoprim/sulphonamide combinations, which have a particular time component to their killing activity and limited **post-antibiotic effect (PAE).** The PAE is the time taken for the bacteria to start growing again once the antimicrobial concentration has fallen below the inhibitory concentration and is usually a matter of hours. The AUC/MIC ratio can also be applied to co-dependent antibiotics, which are often bacteriostatic antimicrobials, such as the tetracyclines, macrolides and pleuromutilins and have both time and concentration effects on killing.

Figure 1.5. Basic pharmacokinetic parameters used following a bolus dose or oral dose of an antimicrobial via water or feed (*Burch 2009)



Usually, the MIC needs to be replaced by the minimum bactericidal concentration (MBC) for bacteriostatic drugs to achieve the right ratios, as their MBC/MIC ratios can be substantially higher than 1-2. For immuno-intact animals and man the optimal kill can be as low as an AUC/MIC of 50, whereas in immuno-compromised human patients AUC/MIC ratios have been increased to 200 to obtain control of mutants (Tam *et al*, 2005). The AUC/MIC relationship can usefully be employed for orally administered products given in feed or drinking water. An additional useful parameter is the **steady state concentration (Css)**. After feed or water medication, the plasma concentration is comparatively flat compared with an injection. A steady state can be calculated by dividing the AUC_{24h} by 24h and the figure should be between 4-5 times the MIC for a bactericidal effect or 1 times for a bacteriostatic effect. It has also been found applicable to antimicrobial concentrations in the gut and the treatment of intestinal infections by the author but **faecal (protein) binding** and **pH** also may have an influence. Some

antibiotics, like the tetracyclines also bind to minerals like calcium, potentially reducing their bioavailability.

Time above the MIC is important not only for antimicrobials like penicillins and cephalosporins but also for determining inter-dose timing; e.g. should a drug be given once or twice a day? Again **dosage interval** is not so important for products administered orally to the pig either in feed or water, as it is often dependent on the system involved and the frequency of feeding and drinking. Most water and many feed systems are on an *ad-libitum* basis.

The method by which the **concentration of the antimicrobial** is determined is also important. High pressure liquid chromatography **(HPLC)** often with mass spectroscopy (MS) determines the concentration of a specific substance. However, many antimicrobials break down to still antimicrobially active metabolites, e.g. enrofloxacin breaks down to ciprofloxacin and therefore both substances need to be tested. In some cases therefore, the older method of **microbiological assay** gives a more representative result of total bioactivity of an antimicrobial substance. Many of the older trials were carried out using microbiological assays. Although they do not specifically define, which substance is bio-active, which is a disadvantage, often the system is carried out using serum or plasma and automatically takes into account such variables as **protein binding** of the drug, which is an additional factor when using the activity of a substance in a model or for integration.

Determining antimicrobial concentrations at sites of infection, other than in plasma is sometimes quite difficult, hence the reliance on **plasma concentrations as an indirect surrogate marker**, as it drives the concentration at other sites, such as extracellular fluids or the extracellular compartment. Some pharmacologists have tried to use tissue concentrations e.g. bronchial mucosa and colon mucosa but generally, if the organism is outside the cell or in the gut, they do not reflect likely antibacterial effect. The author (DB) has found gut contents concentration to be a representative PK parameter from this work, while others have used transudate and exudate models to determine likely antimicrobial concentrations and effect on bacteria in infected models (Lees and Aliabadi, 2002). More recently Croneberger *et al* (2009) described the use of a microdialysis method to determine the concentration of cefpirome in the extracellular fluid of the lung and the non-protein bound fraction in anaesthetized pigs, to determine more accurately the concentration at the site of infection. There is only limited data of this nature available, hence the use more commonly of plasma concentrations.

Pharmacodynamics

Pharmacodynamics are the action of a drug on an organism and is usually represented by the minimum inhibitory concentration (MIC) of the antibiotic; the concentration, which stops the bacterium growing. In contrast, the pharmacokinetics reflect the concentration of the drug achieved in a target tissue, usually blood/plasma over time. There are limitations from the MIC data as it does not necessarily describe the killing concentration (minimum bactericidal concentration (MBC) or rate of killing of the

antimicrobial drug being examined. The relative importance of this parameter is demonstrated in the following chapters.

There are various methods to determine the antimicrobial susceptibility of a bacterium to an antimicrobial substance. The most common method is to use the MIC system using doubling dilutions of the antimicrobial and determining the lowest concentration of the drug to inhibit the growth of the bacterium. This is commonly carried out in a test tube system, where as the organism grows, cloudiness appears in the solution. Marker substances can be added to show a change of pH, should the organism grow. If there is no growth the marker does not change colour; this method is commonly employed for *Mycoplasma* spp. Agar plates, containing the antibacterial, can also be used and the prevention of colony growth or haemolysis in blood agar plates can also be a useful marker.

In diagnostic laboratories, the Kirby-Bauer method of growing the organism on a plate and using antimicrobial sensitivity discs with specified concentrations of the antibiotic in the disc. Whether there is growth of the bacterium around the disc or not is recorded and the diameter of the zones of inhibition can be measured. When the methods are carried out according to the Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee of Clinical Laboratory Standards – NCCLS) guidelines (Anon., 2002) then an estimation of the likely MIC can be made, related to the size of the diameter of the zone of inhibition. Although the results can be variable, it is a useful assessment in less sophisticated laboratory settings.

An Etest® method (Product literature – Biomerieux S.A.) can be used, where a small piece of paper with increasing concentrations of antibiotic are marked on the paper. Where the bacterial growth inhibition coincides with the concentration on the paper it indicates the approximate MIC.

Increasingly, the author could find flaws in the methods used to develop the pharmacodynamic data, which caused major variations in the MIC data. This made PK/PD integration difficult to interpret.

Inoculum strength of the bacteria can interfere with the MIC. The higher the inoculum, the higher the MIC will be. The methods established by CLSI are good for growing the bacteria and give a consistent result but may interfere with the activity of the antimicrobial e.g. if it is pH sensitive. This became apparent for *A. pleuropneumoniae* for example and certain antimicrobials. Stability of the antimicrobial in solution, especially over several days culture e.g. chlortetracycline, is also important for the more slow growing organisms like mycoplasma. Some intracellular obligate organisms like *L. intracellularis* are very difficult to grow and cell culture type, concentration range and endpoint determination may have an impact on MIC determination.

There is limited published data available on MBCs for primarily bacteriostatic antibiotics and very little data on killing curves, which is concentration and time dependent and could have an impact on duration of treatment.

Susceptibility patterns, where populations of a bacterial species are arranged by MIC of an antibiotic, were shown to give useful insights how resistance has developed over time in these populations and often corresponds to the antibiotic's usage. The main driver for the pattern selection to develop appeared to be the effective therapeutic antibiotic concentration rather than sub-inhibitory concentrations and examination of this phenomenon is frequently supported by the drug's pharmacokinetics and also forms a major part of this thesis.

Fundamentally, the author found PK/PD principles or relationships appear to apply in pig medicine, as in man, especially for injectable products, which was the original basis in human medicine. The examination of these principles and relationships for infections in the gut and respiratory tract of the pig, form the basis of this thesis. The variations between the different modes of action of the antimicrobials and the sites of infection have been established, where possible, especially for the bacteriostatic drugs such as the tetracyclines, macrolides and pleuromutilins, which are so important in pig medicine. The integration of the PK/PD relationships with clinical or bacteriological response, can offer an explanation of what is going on and can be used in the future to determine optimum dose rates to control infections. It is hoped with better, application of these PK/PD principles, antibiotics will be used more efficiently and as well as clinical response improvements, the development of antimicrobial resistance might be reduced.

1.6 Antimicrobial resistance overview

Although antimicrobial resistance and specifically resistance mechanisms were not considered a key part of this thesis, as it was primarily an exploration of the PK/PD relationships of various antimicrobials against susceptible organisms commonly found in the pig. This was to confirm PK/PD principles that had been derived originally from human medicine. It became clear that a section on this subject might be helpful to the understanding of the veterinary practitioner, on how antimicrobial drugs work and how their use might be associated with resistance development. Antimicrobial resistance development has become a major issue with many of the antimicrobial drugs commonly used in veterinary medicine and has a major impact on drug selection for treatment and their efficacy.

Antimicrobial resistance development (Based on Giguere *et al*, 2006)

Antimicrobial resistance and its development is a complex subject. Originally, it was a natural defence mechanism of bacteria, to fight against naturally occurring antibiotics, which were produced by a number of fungi and bacteria in the wild to aid their survival. Antibiotics are now produced commercially by fermenting these fungi and bacteria and extracting the antibiotic (e.g. penicillins, tetracyclines, aminoglycosides) or frequently adding side chains and producing semi-synthetic antibiotics (e.g. amoxycillin, methicillin, cephalosporins) with different or improved spectra of activity, pharmacokinetics or efficacy. There are also fully synthesized antimicrobial compounds, like the sulphonamides, trimethoprim and the fluoroquinolones. Each antimicrobial family and its sub-groups has its own mode of action and thereby each bacterial or mycoplasmal species develops its own way of countering the antimicrobial, as a defence

or resistance mechanism, so that it can survive and continue to live in the environment it inhabits e.g. the gut, the respiratory tract etc. The main antimicrobial families, their mode of action and common resistance mechanisms are summarised in Table 1.6.

Simply, the bacterium is constructed of an outer cell wall of variable thickness with an inner cell membrane. It has chromosomal DNA in a tightly coiled chain, which controls growth and multiplication. The DNA sends messages to the ribosome (rRNA 50S subunit and 30S subunit) via messenger RNA (mRNA) to produce polypeptides or proteins for growth. Transfer RNA (tRNA) carries the amino acids to the ribosome to form the new proteins. When the bacterium is ready to divide the DNA uncoils and divides and a new bacterial cell is formed. Some bacteria multiply rapidly, like *E. coli* and some grow slowly like *Brachyspira* spp. The rapid, prolific growers have more of a chance to develop new **DNA mutants** and these mutations may increase resistance to antibiotics. All the bacterial structures can be targets for antimicrobial attack. The penicillins or beta-lactam antibiotics target the cell wall, the polymixins the cell membrane, the fluoroquinolones the DNA and the tetracyclines, macrolides, pleuromutilins, aminoglycosides the RNA.

Bacteria are routinely classified as **Gram positive** (blue staining with Gram stain - due to a thick cell wall) these include *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus* spp and *Clostridium* spp. **Gram-negative** (pink staining – thin cell wall) bacteria are primarily found in the gut, such as *E. coli, Salmonella* spp, or in the respiratory tract *A. pleuropneumoniae, P. multocida* and *H. parasuis*. They are further divided into **aerobic** (need oxygen to survive) or **anaerobic** where they do not use oxygen and have different metabolic pathways. *Enterococcus* spp and *Clostridium* spp are examples of Gram +ve anaerobic bacteria and are found in the large intestine or colon and *Brachyspira* spp are examples of Gram –ve anaerobic bacteria, also found in the colon. Some bacteria can live in both environments, like *E. coli*. The commonly monitored bacteria for public health and regulatory resistance monitoring are the **commensal bacteria**, such as *E. coli* and *Enterococcus* spp, and *Salmonella* spp (mainly *S.* Typhimurium in pigs) and *Campylobacter* spp (mainly *C. coli* in pigs) for potential **zoonotic infections**, those infections in animals that cause disease in man.

Resistance mechanisms

When antimicrobial resistance is examined there are some other key factors to consider. Some bacteria are intrinsically resistant to certain antibiotics, usually due to their mode of action. For example penicillins, which act on the cell wall of a bacterium, are not effective against *Mycoplasma* spp, as they do not have a cell wall, only a cell membrane. Macrolides, like tylosin, cannot penetrate the cell membranes of certain Gram -ve bacteria like *E. coli*; aminoglycosides work poorly against anaerobic bacteria, as they use an oxygen-dependent mechanism to penetrate the bacteria. Susceptible bacteria can acquire resistance by a variety of mechanisms: - prevention of an antimicrobial substance reaching a target by reducing its penetration into the bacterial cell often via porin changes, as they are often large molecules; the use of a general or specific efflux-pump mechanism that expels antimicrobial agents from the bacterial cell; inactivation of the antimicrobial agent by modification or degradation either before or after penetrating

the cell e.g. beta-lactamases; modification of the antimicrobial target so that it cannot act on it, or the microorganism's activation or acquisition of an alternative pathway rendering the target dispensable e.g. trimethoprim (summarised in Table 1.6).

Acquired resistance can be achieved by a number of mechanisms, which are usually the result of selection pressure from the use of antibiotics. **Mutations** in the chromosomal DNA, which then alter the DNA coiling etc, are important for the fluoroquinolones. DNA changes, which affect the binding sites on the ribosomes, are important for the macrolides, lincosamides, streptogramins and pleuromutilins and co-resistance can occur between these families, as their sites of action often are close or overlap on the 50S subunit. The acquisition of resistance genes from outside the bacterial cell is also highly important. Some bacteria pick up extraneous DNA genes from other broken down cells by **transformation** and insert them into the chromosome. Others receive DNA into the chromosome via **transduction** from viral bacteriophages but the most common route is via **plasmid transfer** at **conjugation** of two cells. The plasmid can be independent of the chromosome in the cell and made up of a variety of DNA genes or open-reading frames (ORFs), which may be functionally significant or not. Plasmids can often carry multiple-resistance genes, which are often carried in **transposons** or **integrons**, which are sections of genetic material that can insert themselves via enzymes transposases and integrases, respectively, usually into plasmids but also into the chromosome of a bacterium. This is a very common route of resistance transmission between enteric bacteria, like *E. coli*; hence they are good indicators for monitoring resistance.

Table 1.6. Antimicrobial family, mode of action and common resistance mechanism (*Burch, 2011)

Antimicrobial family	Mode of action	Resistance mechanism
Beta-lactam antibiotics Penicillins: Penicillin G, penicillin V Methicillin, oxacillin Ampicillin, amoxycillin Piperacillin Cephalosporins: *1st and 2nd generation* Cephalexin, cephradine *3rd and 4th generation* Ceftiofur, cefquinome Cefotaxime, ceftazidime (human use) Monobactams: Aztreonam Carbapenems: Imipenem, meropenem *Beta-lactamase inhibitors* Clavulanic acid, sulbactam tazobactam	Inhibit cell wall production. Binds enzymes (PBPs) which help form peptidoglycans. Inhibits/binds to beta-lactamase enzymes	-Beta-lactamase production primarily – *bla* genes. -Changes cell wall protein enzymes so that they cannot bind to PBPs. *MecA* gene for methicillin resistance – *S. aureus*. -TEM-1, TEM-2, SHV-1 type beta-lactamase (*bla*) producing genes - plasmid transfer usually -Cephalosporinases -Extended-spectrum beta lactamases (ESBLs) CTX-M beta-lactamase (*bla*) genes – plasmid transfer usually (better term - expanded spectrum cephalosporinases - ESCs) -Carbapenemases - KPC, CMY (*bla*) genes – serine based; IMP and VIM – metallo beta-lactamase (*bla*) enzyme genes (Zn dependent) -AmpC gene (bla_{CMY} sub-group) – cephalosporinase; Metalloenzyme genes – inhibitor resistant genes *mecA* gene - cannot bind to PBPs;
Polymixins Colistin	Action on cell membrane – disrupts permeability	Unclear – decreased bacterial permeability
Tetracyclines Chlortetracycline, oxytetracycline	rRNA – binds to 30S subunit and interferes with amino acid transfer	Inducible efflux in *E. coli* etc (*tetA, tetB, tetC*) Binding site changes (*tetO, tetM* genes) Rare, changes to tetracycline molecule

Antimicrobial family	Mode of action	Resistance mechanism
Doxycycline, minocycline	Prevents protein production	
Aminoglycosides Streptomycin Neomycin, Kanamycin Apramycin, gentamicin Amikacin **Aminocyclitol** Spectinomycin	rRNA – binds to 30S subunit, so misreads genetic code. Prevents protein production. Effect on cell membrane permeability	Phosphorylation, adenylation and acetylation of aminoglycoside (*aph, aad, aac* genes) stops them binding. Streptomycin – single binding site Others – multiple binding sites, slower resistance, primarily plasmid
Macrolides/azalides (M) Tylosin, tylvalosin, tilmicosin (16C) Tulathromycin (15 & 13C) Azithromycin (15C) Erythromycin (13C)	rRNA – binding to 50S subunit. Inhibits transpeptidation. Prevents protein production	Methylation of rRNA in G+ve orgs (*ermA, ermB, ermC* genes) inhibits binding. Co-resistance possible (*mlsB*). Active efflux (*mef* gene) Enzymatic inactivation possible
Lincosamides (L) Lincomycin, clindamycin	rRNA – binding to 50S subunit. Inhibits peptidyl transferase. Prevents protein production	Methylation of 23S subunit of rRNA, prevents binding. Co-resistance possible (*mlsB*). Drug inactivation possible
Streptogramins (S) Virginiamycin	rRNA – binding to 50S subunit. Prevents protein production A and B class	Methylation of rRNA in G+ve orgs Class A – active efflux and drug inactivation (*vgaA, vgaC, msrA* genes) Co-resistance to S, M, L and P. Class B – methylation of 23S subunit of rRNA (*erm* genes)
Pleuromutilins (P) Tiamulin, valnemulin	rRNA – binding to 50S subunit. Inhibits peptidyl transferase. Prevents protein production	Chromosomal mutations – stepwise Methylation of rRNA in G+ve orgs Co-resistance genes (*vgaA, vgaC*)
Chloramphenicols Thiamphenicol, florfenicol	rRNA – binds irreversibly to 50S subunit. Inhibits peptidyl transferase. Prevents protein production	Acetylation of drug in enterobacteria (*catA* gene) prevents drug binding. Plasmid transmission. Efflux (*cmlA, floR* genes); mutations at target site and increased permeability barriers
Sulphonamides Sulfadiazine	Purine synthesis for DNA. Interferes folic synthesis	Chromosomal mutations but plasmid and integron-mediated resistance more common. Bypass blocked pathway by resistant dihydropteroate synthetase (*sul1, sul2, sul3* genes)
Diaminopyrimidines Trimethoprim, ormethoprim	Purine synthesis for DNA. Interferes folic synthesis	Bypass blocked pathway by resistant dihydrofolate reductase (*dfr* gene). Often transposon or integron encoded on plasmid or chromosome
Quinolones Nalidixic acid, oxolinic acid **Fluoroquinolones** Flumequine Norfloxacin Enrofloxacin, ciprofloxacin marbofloxacin	Interrupts DNA breakage-reunion step by binding DNA-gyrase or topoisomerase II (subunits GyrA & GyrB) topoisomerase IV (ParC & Par E subunits)	Target modification – DNA gyrase (*gyrA* and *gyrB*) one step resistance + *parC* & *parE* – complete resistance. Nalidixic acid resistance - *gyrA* mutation only Decreased permeability – outer membrane porins mutations (*ompF*) Efflux pumps Resistance primarily clonal but recently found plasmid gene (*qnr*) on integron. Campylobacter only have topoisomaerase II, so one step resistance

Chapter 2. PK/PD relationship analysis and integration for respiratory infections

2.1 Introduction

Respiratory infections are of major significance in pig production and substantial amounts of antimicrobials have been used to control these diseases. In spite of the widespread use of vaccines for enzootic pneumonia (EP), the causal agent *M. hyopneumoniae* is commonly found on pig farms and in international surveys 38-100% of pigs had pneumonic lesions at slaughter (*Guerrero, 1990). *Pasteurella multocida* is commonly associated with *M. hyopneumoniae* infections, as a secondary bacterial invader and *A. pleuropneumoniae* can be a primary pathogen or complicate EP. The other important bacterial respiratory infection is *H. parasuis*, the cause of Glässer's disease, which causes both a respiratory and a bacteraemic disease with serious secondary effects such as peritonitis, pericarditis and infectious arthritis.

This chapter will be divided into two sections. The first section will deal with the oral antimicrobial drugs that are used primarily for treating *M. hyopneumoniae*. Their pharmacokinetics and pharmacodynamic properties will be reviewed and compared to establish suitable assessments for both bactericidal and bacteriostatic drugs. The second section will look at PK/PD integration for the treatment of the bacterial respiratory infections and compared with mycoplasmal infections. The impact of drug concentrations in lung and leucocyte concentrations will also be assessed.

2.2 *Mycoplasma hyopneumoniae* infections – PK/PD integration

Introduction

Enzootic pneumonia (EP) is in most cases a chronic respiratory disease caused by *M. hyopneumoniae.* It has a widespread occurrence in most pig populations of the world, commonly causing characteristic lung lesions in 40-50% of all slaughter pigs. It usually causes a relatively mild disease, as a sole pathogen, characterised by occasional coughing, depression in growth rate and feed conversion efficiency (FCE) and a low mortality rate. However, in naïve herds, undergoing primary breakdown, enzootic pneumonia can be more severe. Commonly, field infections are complicated by secondary bacterial invaders and viruses, which render the disease both more severe and more difficult to control. Antimicrobial drugs were widely used prophylactically, prior to the introduction of *M. hyopneumoniae* vaccines, to attempt to prevent disease development. Drugs are still commonly used but often more strategically at times when an increase in respiratory disease is expected, for example after moving and mixing or for treatment when acute clinical disease is present. The disease often involves a combination of mycoplasmas, bacteria and viruses, causing the porcine respiratory disease complex (PRDC). *M. hyopneumoniae* is considered of major significance in PRDC.

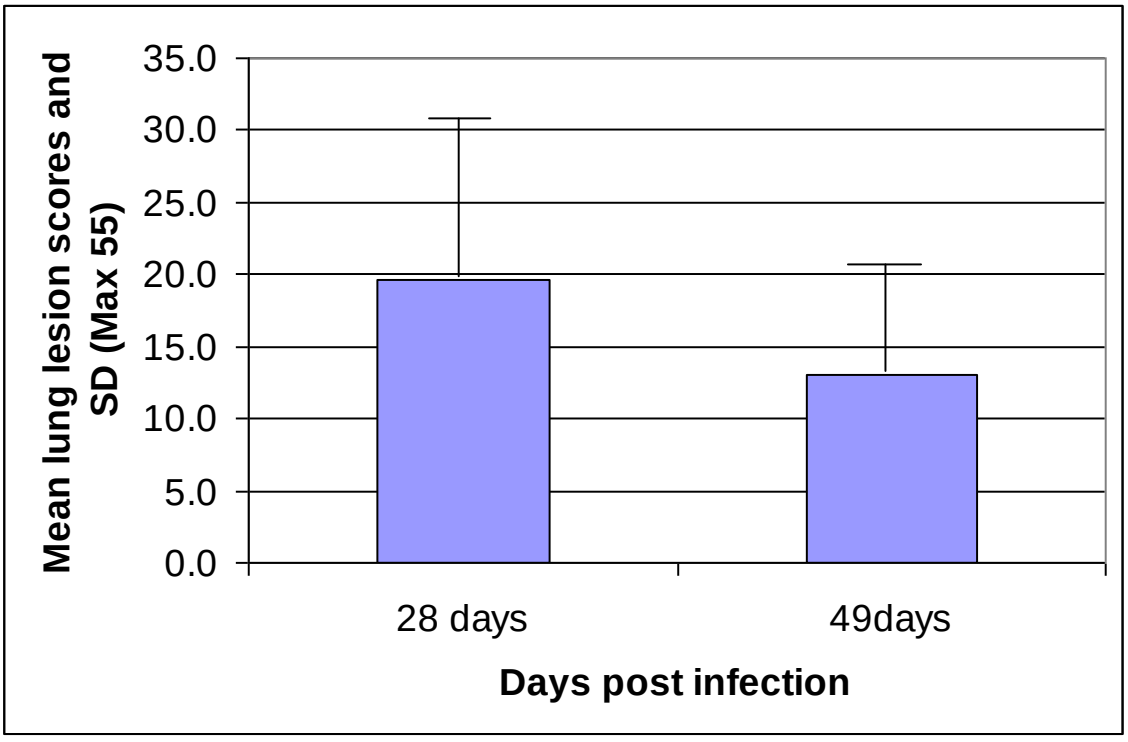
The aim of this section is to compare the *in vitro* activity of several antimicrobial drugs against *M. hyopneumoniae* with their efficacy in infection models of EP and, where data are available, examine their pharmacokinetic/pharmacodynamic (PK/PD) relationships with their efficacy.

Characteristics of enzootic pneumonia

Whittlestone (1973) described the progressive development and resolution of enzootic pneumonia lesions induced either by infection with *M. hyopneumoniae* or ground lesion suspensions administered intranasally. Early gross lesions could be detected approximately 7-10 days after infection. These continued to develop and establish between 17-40 days and clinical recovery and resolution of lesions took 69–262 days.

Goodwin (1979) also demonstrated this protracted disease characteristic in an artificial challenge study, under non-intensive conditions, in which pigs were killed at 28 and 49 days after infection. The lesions were scored and shown to be starting to regress at 7 weeks, although they were still described as active at that time (see Figure 2.1).

Figure 2.1. Enzootic pneumonia lesion development and regression in an artificial challenge model (Goodwin, 1979)



The lesions affect primarily the ventral parts of the lung lobes and may affect the cranial, middle and intermediate lobes and relatively small anterior portions of the large caudal lobes. Up to 55% of the whole lung can be affected with enzootic pneumonia, in the most severe cases. Following infection, the organism progressively colonises down the respiratory tract and can be identified mainly on the bronchiolar surfaces where there is

depletion of cilia. *M. hyopneumoniae* is closely associated with the epithelial cell surface. There is peribronchiolar and perivascular, lymphoreticular hyperplasia and progressive development of alveolar-cell pneumonia. There appears to be a marked lymphoreticular response to the mycoplasmas but the clearance of the organism is very slow, suggesting immuno-defence interference. As a result, resolution of the lesions is a protracted but ultimately, an effective immunity is established and pigs are resistant to subsequent challenges by *M. hyopneumoniae*.

Secondary bacterial invaders are very common, especially *P. multocida,* due to the reduced clearance and immunological defence processes. It has been shown that simultaneous *P. multocida* infections can double the lung lesion size caused by *M. hyopneumoniae* alone (*Ciprian *et al*, 1986). Enzootic pneumonia also appears to enhance other bacterial infections such as those caused by *A. pleuropneumoniae.* With the spread of virus-associated diseases, such as porcine reproductive respiratory syndrome (PRRS) virus and post-weaning multisystemic wasting syndrome (PMWS) associated with porcine circovirus type 2 (PCV2), there has been an increase in associated bacterial infections, especially *H. parasuis* and *S. suis* and almost all of the lobes of the lung can be affected with pneumonia.

Assessment and scoring of the gross lung lesions has been the basis for the evaluation of the efficacy of both antimicrobial drugs and vaccines. A number of methods have been described, but the most common are by visual assessment and scoring, which is rapid and relatively consistent. Three methods are in common use and there are similarities between them.

Table 2.1. Comparison of some pneumonic lung lesion scoring methods

Lung lobe	Goodwin *et al* (1969) Max score per lobe	Percentage system - max score per lobe (Unknown source)	*Thacker *et al*, (1988) – adjusted percentage max score based on size of lobe
L cranial (apical)	10	10	4 (100 x 0.04)
L caudal segment (cardiac)	10	10	9 (100 x 0.09)
L caudal (diaphragmatic)	5	25	25 (100 x 0.25)
R intermediate	5	10	5 (100 x 0.05)
R cranial (apical)	10	10	7 (100 x 0.07)
R middle (cardiac)	10	10	15 (100 x 0.15)
R caudal (diaphragmatic)	5	25	35 (100 x 0.35)
Total max.	**55**	**100**	**100 (100 x 1)**

The Goodwin *et al* (1969) method gives maximum score of 55 and is approximately equivalent to 55% of the lung being involved. It is adequate for EP, as it primarily affects the cranio-ventral aspects of the lobes, especially the cranial lobes and rarely goes further in uncomplicated situations into the caudal diaphragmatic lobes. The percentage method is similar, very rapid to do, especially when working on a slaughter line, but takes into account the whole lung rather than just the normally EP-affected

parts. This has become important with the upsurge of virus infections (PRRSV and PCV2) where up to 80% of the total lung can be a consolidated lesion. The *Thacker *et al* (1988) method is a more accurate approach to take into account the relative differences in the sizes of the lobes and gives them a weighting but is more time-consuming.

In the efficacy trials, percentage lung lesion reductions in comparison with the untreated control will be used, so that it takes into account the differing methods of assessment, but enables an overall comparison to be made.

Comparative antimicrobial activity (pharmacodynamics)

Several authors have reported on the susceptibility of reference and field isolates of *M. hyopneumoniae* (Inamoto *et al*, 1994; Hannan *et al*, 1997; Aitken *et al*, 1999; *Thongkamkoon *et al*, 2002; Vicca *et al*, 2004) to several drugs. All used similar methods of broth dilution to determine the minimum inhibitory concentrations (MICs). The determination of the MIC is the lowest concentration of antimicrobial, which prevents the colour change in the pH-dependent colour marker system (phenol red), associated with the fermentation of sugars in the broth. Culturing is normally continued for 5 days as *M. hyopneumoniae* is a relatively slow growing organism. Some authors have used reference strains, usually the type 'J' strain, also referenced as NCTC10110. These are useful for direct comparative purposes of the investigator's methods and results. The references are summarised in Table 2.2 and the results are compared in Table 2.3.

Table 2.2. References used and origin of isolates and number of isolates

Reference	Year	Country	No of ref strains	No of field isolates
1. Inamoto *et al*	1994	Japan	1	25
2. Hannan *et al*	1997	Worldwide	1	20
3. Aitken *et al*	1999	UK	-	10
4. *Thongkamkoon *et al*	2002	Thailand	1 (not reported)	27
5. Vicca *et al*	2004	Belgium	1	21
6. Godinho	2008	EU	1	27

Table 2.3. Comparison of MIC50, MIC90 and Range (µg/ml) for antimicrobials drugs against *M. hyopneumoniae* by reference

Antimicrobial	Reference	Ref strain MIC	MIC50	MIC90	Range
Chlortetracycline	1 4	12.5 -	12.5 0.39	≥100 1.56	0.78-≥100 <0.024-3.125
Oxytetracycline	1 2 3	0.78 0.25 -	0.78 0.25 0.078	6.25 1 0.31	0.1-12.5 0.025-1 0.039-0.63

Antimicrobial	Reference	Ref strain MIC	MIC50	MIC90	Range
	5	0.12	0.12	1.0	0.03-2.0
Tiamulin	1	≤0.0125	≤0.0125	0.05	≤0.0125-0.05
	2	0.025	0.05	0.05	0.01-0.1
	3	-	0.039	0.078	0.039-0.16
	4	-	0.006	0.048	<0.006-0.097
	5	0.03	≤0.015	0.12	≤0.015-0.12
Valnemulin	3	-	0.0024	0.0049	0.0024-0.0098
	4	-	<0.006	<0.006	<0.006
Tylosin	1	≤0.0125	0.025	0.05	≤0.0125-0.1
	2	0.025	0.1	0.25	0.025-0.25
	3	-	0.16	0.31	0.16-0.63
	5	0.03	0.06	0.5	≤0.015->1.0
Tilmicosin	1	0.1	0.1	0.39	≤0.0125-0.39
	4	-	0.39	1.56	<0.024-3.125
	5	0.25	0.25	0.5	≤0.25->16
Tylvalosin	1	≤0.0125	≤0.0125	≤0.0125	≤0.0125-0.05
Josamycin	4	-	0.048	0.097	<0.006-0.195
Tulathromycin	6	-	0.03	0.06	≤0.004-0.125
Lincomycin	1	0.025	0.025	0.1	≤0.0125-0.39
	3	-	0.31	0.63	0.31-0.63
	4	-	0.048	0.097	<0.006-0.39
	5	≤0.06	≤0.06	≤0.06	≤0.06->8.0
Enrofloxacin	2	0.05	0.025	0.05	0.01-0.1
	5	0.03	0.03	0.5	0.015->1.0
Danofloxacin	2	0.025	0.025	0.05	0.01-0.05

Most antimicrobial drugs used in pigs to treat respiratory infections, including the pleuromutilins, macrolides and the fluoroquinolones, have relatively low MICs and it seems probable that only low levels of resistance have developed as reported by Vicca *et al* (2004). In the case of the tetracyclines, the MICs tend to be higher, especially for chlortetracycline in Japan, but less so for oxytetracycline and may reflect their extensive use over many years.

Variations in the MIC occur depending on the media used and agar plates normally yield higher values than broth. High initial organism inoculum count also tends to increase the MIC. The pH can impact the activity of some drugs; the initial pH is 7.8. A slightly alkaline condition benefits tiamulin, but may have a negative impact on chlortetracycline activity. Stability of the substance e.g. chlortetracycline, in the broth is also important, especially over prolonged culture. The endpoint, i.e. either at the first colour change of the control or after a further day or so of culture, can also affect the MIC result.

Comparative antimicrobial efficacy

A number of challenge studies have been reported as new drugs have been developed. However, there are relatively few comparative studies involving several products.

The majority of studies have used EP lung lesion homogenate inoculated intra-nasally, (occasionally intra-tracheally), either on one or two occasions, to induce the infection. Occasionally, pigs are naturally infected. The EP infection is often superimposed by a bacterial infection, either as part of the challenge or by a contaminant of the homogenate, or natural infection in the pig. This contamination may compound interpretation of the results. In prevention studies, medication usually begins just before, or at the time of infection, and in treatment studies it commences 10-28 days after infection when the lesions have started to develop.

Data from the studies are summarised in Table 2.4 and the percentage lung lesion reductions are presented in Table 2.5.

Table 2.4. Summaries of EP model infections by reference

Trial reference	Drug	Route of administration	Prevention (days)	Treatment (days)	Infection route	Bacteria isolated
1.*Thacker *et al* (2000)	Chlortetracycline	In feed	-3 to +10 Term 28	10 to 24 Term 28	I/T	-
2.Schuller *et al* (1977)	Tiamulin	In feed	-3 to +7 Term 35	-	I/N	Mh
3.Goodwin (1979)	Tiamulin	In feed	-	28 to 37 Term 49	I/N	-
4.Hannan *et al* (1982)	Tiamulin Tylosin	Gavage b.i.d.	-	14 to 24 Term 38	I/N	Mh, Ag, Ph, Pm
5.*Simon *et al* (1990)	Tiamulin Enrofloxacin	In feed	-	0 to 10 Term 15 & 29	Natural	Yes – NR
6.*Miller and Stipkovits, (1991)	Tiamulin + chlortetracycline Tylosin + sulphadimidine	In feed	0-28 Term 28	-	I/N	Pm, Bb
7.Stipkovits *et al* (2001)	Tiamulin + chlortetracycline Valnemulin + chlortetracycline Tilmicosin Lincomycin + chlortetracycline	In feed	-	9 to 21 Term 22-24	I/N aerosol Mh – 0 Pm – 8 App – 15	Pm, App

Table 2.4 continued on next page

References with an asterisk * are not refereed

Table 2.4 (contd.) Summaries of EP model infections by reference

Trial reference	Drug	Route of administration	Prevention (days)	Treatment (days)	Infection route	Bacteria isolated
8.*Morgan *et al* (1996)	Valnemulin	In feed	0 to 21 Term 21	-	I/N	-
9. *Burrows *et al* (2002)	Valnemulin Tylvalosin	In feed	- -1 to +6 Term 19	4-11 4-11 Term 19	I/N	-
10. *Yamamoto *et al* (1986)	Josamycin	In feed	-1 to +3-+7 Term 36	-	I/N x2	-
11.*Kubo *et al* (1990)	Lincomycin	In feed	0 to 7 Term 27	-	I/N x2	-
12. *Ross *et al* (1990)	Danofloxacin	In water	-2 to +10 Term 10 & 20	-	I/T	-
13. *Kuwano *et al* (1992)	Ofloxacin	In feed	0 to 7 Term 35	-	I/N	-
14. Vicca *et al* (2005)	Tylosin	In feed	-	13-33 Term 33	I/T	-
15. McKelvie *et al* (2005)	Tulathromycin Enrofloxacin	Injection	-	5 Term 12-13	I/N	-

Key: I/N = intra-nasal; I/T = intra-tracheal; Mh = *M. hyorhinis*; Ag = *Acholeplasma granularum*;
Pm = *P. multocida*; Ph = *P. haemolytica*; App = *A. pleuropneumoniae*; Bb = *Bordetella bronchiseptica;*
b.i.d. = twice daily

References with an asterisk * are not refereed

Table 2.5. Comparison of antimicrobial efficacy judged by lung lesion reduction (%) for the prevention and treatment of enzootic pneumonia under controlled conditions

Antimicrobial	Ref.	Dose (mg/kg bwt/day)	Inclusion rate in feed (ppm)	Prevention Lesion reduction (%)	Treatment Lesion reduction (%)
Chlortetracycline (CTC)	1	22	550ppm	95	36
Tiamulin	2	5	100ppm	35	-
		10	200ppm	58	-
	5	10	200ppm	-	92 (day 15)
					57 (day 29)
	4	20	Gavage b.i.d.	-	98
	3	50	1000ppm	-	69
Tiamulin + CTC	6	5 + 15	100 + 300	97	-
	7a	2 + 22	38.5 +440	-	76
	7b	5 + 20	100 + 400	-	76
Valnemulin	8a	10	200	45	-
		15	300	43	-
		20	400	71	-
	8b	10	200	79	-
	9	10	200	-	38
Valnemulin + CTC	7a	1.25 + 22	25 + 440	-	90
		2.5 + 22	50 + 440	-	93
	7b	1.25 + 20	25 + 400	-	86
		3.75 +20	75 + 400	-	90
Tylosin	4	100	Gavage b.i.d.	-	95
	14	5	100		67
Tylosin + sulphadimidine	6	5 + 15	100 + 300	52	-

Table 2.5. continued on next page

Table 2.5 (contd.) Comparison of antimicrobial efficacy, judged by lung lesion reduction (%) for the prevention and treatment of enzootic pneumonia under controlled condition

Tylvalosin	9	2.5 2.5 5	50 50 100	45 - -	- 51 32
Josamycin	10	2.5 5	50 100	12 70	- -
Tilmicosin	7b 7a	15 20	300 400	- -	60 60
Lincomycin	11	4.4 8.8	88 176	26 41	- -
Lincomycin + CTC	7b	5 + 20	100 + 400	-	24
Enrofloxacin	5	7.5 10	150 200	- -	92 (day 15) 92 (day 15) 79 (day 29)
Danofloxacin	12	3.4	W25	94 (day 10) 86 (day 20)	- -
Ofloxacin	13	0.6 2.5 10	12.5 50 200	51 82 100	- - -
Tulathromycin Enrofloxacin	15	2.5 5.0 for 3 days	-	- -	49 90

Key: W = in water; G = by gavage; b.i.d = twice daily; a / b – refer to separate trials within the one reference

Chlortetracycline was highly effective in the prevention of EP, which was also the author's own findings (Burch and Morgan – unpublished data) but less efficacious for the treatment of EP. Combinations of CTC with the pleuromutilins were highly efficacious for both the prevention and treatment of EP complicated with bacteria.

Tiamulin and valnemulin both showed dose/response effects for the prevention of EP and also a good response was achieved with a second strain more sensitive to valnemulin. Tiamulin at high levels (20mg/kg bodyweight – twice the recommended in-feed dosage levels) was also very effective in treating EP. Tylosin at very high levels of 100mg/kg bodyweight (20 time recommended in-feed dosage rates) administered by gavage was effective against an organism with an MIC of 0.5µg/ml (approximately MIC 90) and recently Vicca *et al*, (2005) showed that 5mg/kg/day given in feed was also effective, but the MIC for the organism was <0.015µg/ml, at the lowest end of its MIC range. A related compound, tylvalosin, showed an intermediate effect at 2.5mg/kg bodyweight for both prevention and treatment. The pleuromutilins, macrolides and lincosamides showed only intermediate preventative effects in comparison with chlortetracycline and the fluoroquinolones. Enrofloxacin was very effective in the treatment of EP and danofloxacin and ofloxacin (a compound originally produced in Japan) were very effective for prevention. Interestingly, the lesion reduction was not maintained with time after treatment in many cases, suggesting new lesion development once medication had been withdrawn, presumably because the mycoplasma started to grow again and complete immunity had not been fully established during the treatment and observation period. In a comparative study using enrofloxacin by injection and tulathromycin, a new triamilide (macrolide related) drug in a long acting formulation injection (McKelvie et al, 2005), the fluoroquinolone reduced lung lesions by 90% and the more bacteriostatic acting tulathromycin by only 49%.

Comparative pharmacokinetic information

Available antimicrobial pharmacokinetic data have been collated from a number of sources but are not always complete. Lung concentration data and correlating plasma levels are presented, where available, for the various antimicrobials. In-feed administration of antimicrobial drugs can have a detrimental effect on concentrations achieved in plasma if the substances are metabolised and excreted rapidly by the liver, reducing their apparent bioavailability. Many compounds can still be detected in the lung, when plasma concentrations are below the limit of detection of the analytical method, because they concentrate there, due to positive diffusion gradients, e.g. the macrolides and pleuromutilins. The substances excreted substantially by the kidneys such as the tetracyclines, lincomycin and the fluoroquinolones usually have detectable blood and lung levels.

Table 2.6. Comparison of blood and lung (homogenate) concentrations achieved by various dosages of antimicrobial (Calculations DB)

Antimicrobial	Reference	Dose (mg/kg/day) estimated	Inclusion level (ppm)	Blood / plasma conc. (µg/ml)	Lung level (µg/g)	Lung / plasma ratio
Chlortetracycline	Asanuma *et al* (1986)	20	400	0.25	0.55	2.2
Tiamulin	*Anderson *et al* (1994) *Ibayashi *et al* (1994)	5.5 11 5.5	110 220 110	<0.3 <0.3 -	1.46 1.99 0.63	>4.9 >6.6
Valnemulin	Anon, Product data	3.75 10	75 200	<0.05 <0.05	0.04 0.23	- >4.6
Tylosin	*Ibayashi *et al* (1994) Hoffman *et al* (1983)	5.5 10 s/c	110 Injection	E<0.04 AUC 16.1	<0.05 AUC 21.3	 1.33
Tylvalosin	*Ibayashi *et al* (1994)	5.5	110	-	0.14	-
Tilmicosin	*Thomson *et al* (1994a, b)	20	400	0.18	1.97	10.9
Lincomycin	*Ibayashi *et al* (1994) *DeGeeter *et al* (1980)	5.5 5.5 11	110 110 220	- 0.16 0.14	0.85 0.66 1.13	- 4.1 8.1
Enrofloxacin	Anon, Product data	5	150	0.24	0.65	2.7
Tulathromycin	Benchaoui *et al* (2004)	2.5	Injection	0.065 mean 168h	1.380 mean 168h	21.2

Key: < = below limit of quantification

Pharmacokinetic/pharmacodynamic relationships

Most classic PK/PD antimicrobial relationships have been based on plasma concentration data, Cmax (maximum concentration reached in plasma after administration) and area under the curve during a 24hour period (AUC24h) and these figures are divided by the MIC. These relationships have been well described by

Toutain (2003). For bactericidal antibiotics such as the aminoglycosides, a figure of 10-12 has been quantified for the Cmax divided by the MIC to give an effective treatment of an infection. For fluoroquinolones, a figure of ≥100h for the AUC/MIC has been proposed for effective bactericidal treatment and <30h for bacteriostasis. For bacteriostatic antimicrobials, similar figures have not been determined. With in-feed or water administration, the intake and absorption of the drug is intermittent and occurs irregularly over a 24h period but the flow and absorption along the gastro-intestinal tract is prolonged. Consequently, plasma and lung concentrations may be relatively constant, in comparison with a bolus dose given either orally or by injection. The AUCs are therefore calculated by either plasma or lung concentration times 24 hours and are summarised in Tables 2.7 and 2.8.

Table 2.7. Integration of antimicrobial drug AUC24h in *plasma* with the MIC50 and MIC90 against *M. hyopneumoniae* (Calculations DB)

Antimicrobial	Feed level (ppm)	MIC50 (µg/ml)	MIC90 (µg/ml)	Plasma conc. (µg/ml)	AUC24h (µg.h/ml)	AUC/MIC 50 (h)	AUC/MIC 90 (h)	Plasma : MIC90 ratios
Chlortetracycline	550	0.39	1.56	0.25	6	15	4	0.16
Tilmicosin	400	0.1	0.39	0.18	4.32	43	11	0.46
Tylosin	110	0.1	0.25	<0.04	<0.96	9.6	3.8	0.16
Lincomycin	220	0.025	0.1	0.14	3.36	134	33.6	1.4
Enrofloxacin	150	0.025	0.05	0.24	5.76	230	115	5.0
Tulathromycin	Injection	0.03	0.06	0.065	1.56	52	26	1.08

Table 2.8. Integration of antimicrobial drug AUC24h in *lung* with the MIC50 and MIC90 against *M. hyopneumoniae* (Calculations DB)

Antimicrobial	Feed level (ppm)	MIC50 (µg/ml)	MIC90 (µg/ml)	Lung conc. (µg/ml)	AUC (µg.h/ml)	AUC/MIC 50 (h)	AUC/MIC 90 (h)
Chlortetracycline	550	0.39	1.56	0.55	13.2	34	8
Tiamulin	220	0.04	0.08	1.99	47.76	1194	597
Valnemulin	200	0.0025	0.005	0.23	5.52	2208	1104
Tylosin	110	0.1	0.25	<0.05	1.2	12	5
Tilmicosin	400	0.1	0.39	1.97	47.28	473	121
Tylvalosin	100	0.0125	0.0125	0.14	3.36	269	269
Lincomycin	220	0.025	0.1	1.13	27.12	1085	271
Enrofloxacin	150	0.025	0.05	0.65	15.6	624	312
Tulathromycin	Injection	0.03	0.06	1.38	33.12	1104	552

From the plasma concentration data, enrofloxacin (fluoroquinolone) achieved an AUC/MIC of over 100h for 90% of the isolates and its therapeutic effect was 79-92% for the treatment of a complicated EP. Three other drugs, tilmicosin, tylosin and tulathromycin, had low AUC/MIC 90 figures, although all of them achieved some effect in the prevention or treatment of EP, especially chlortetracycline with a preventative effect of 95%. Unfortunately, the MIC for the isolate used was not published but subsequently it was reported as 1.0µg/ml (T. Wolff – personal communication). This MIC still does not correlate well with the effect and tends to confirm an over-estimation of the MIC, as a result of the instability of chlortetracycline in culture media over a prolonged time, especially in comparison with oxytetracycline, a closely related compound. Tilmicosin, at the MIC 50 concentration, also showed a marked inhibitory effect, which correlated with an AUC/MIC 50 of 43. Tulathromycin by injection, however, showed an AUC/MIC 90 of 26 which could be classed as an inhibitory level and gave a clinical result, which was inhibitory, with a 49% reduction in lung lesions. The MIC of the isolate used in the study was 0.05µg/ml, which is close to the MIC 90.

With regard to lung concentrations, enrofloxacin achieved an AUC/MIC 90 of 312h, or approximately three times the AUC/MIC 90 figure for serum and this is the approximate ratio lung to blood that is found with this substance. All the other products had high lung AUC/MIC 90 values, except tylosin and chlortetracycline. Valnemulin's lung AUC/MIC figures were particularly high because of the very low MIC values.

In the report of *Morgan *et al* (1996), the MICs of the two *M. hyopneumoniae* used in the studies were given. The effect and lung PK/PD relationships can be examined further.

Table 2.9. Comparison of AUC/MIC lung for valnemulin and reduction in lung lesions (prevention) (*Morgan *et al,* 1996) (Calculations DB)

Trial 1	Valnemulin Feed level (ppm)	Extrapolated lung level* (µg/ml)	AUC lung (µg.h/ml)	AUC/MIC lung (h)	Lung lesion reduction (%)
MIC 0.016(µg/ml)	100	0.12	2.76	173	0
	200	0.23	5.52	345	45
	300	0.35	8.28	518	43
	400	0.46	11.04	690	71
Trial 2					
MIC 0.0078(µg/ml)	200	0.23	5.52	708	79

*Assuming a linear lung concentration with feed concentration.

Valnemulin's AUC lung/MIC of 345 – 518h gave 43-45% reduction in lung lesions and 690-708h gave 71-79% reduction in lung lesions in a prevention trial, suggesting that an AUC lung/MIC of 1000h would be required to achieve complete prevention. This suggests **that lung concentration could be used as a surrogate marker**.

In a study using tiamulin for the treatment of EP, Goodwin (1979) demonstrated a 69% reduction in lesions using 50mg/kg bodyweight or approximately 1000ppm in feed. He also described the MIC of the organism used and the minimum mycoplasmacidal concentration (MMC = MBC). The MIC was 0.11-0.15µg/ml and the MMC was 0.45-0.9µg/ml. In the calculations, an average figure has been used - 0.13 and 0.68µg/ml (MMC/MIC ratio = 5).

Table 2.10. Comparison of AUC Lung/MIC for tiamulin and the MIC and MMC of *M. hyopneumoniae* and lung lesion reduction (Goodwin, 1979) (Calculations DB)

	Tiamulin Feed level (ppm)	Extrapolated lung conc. (µg/ml)	AUC lung (µg/ml/h)	AUC lung /MIC (h)	Lung lesion reduction (%)
MIC 0.13(µg/ml)	1000	9.05	217.2	**1670**	69
MMC 0.68(µg/ml)	1000	9.05	217.2	**319**	69

When the AUC lung is divided by the MMC, a similar AUC/MIC figure to enrofloxacin is achieved (312h), which is a bactericidal antimicrobial. However, the strain used was the reference 'J' strain and the MIC is almost 5-10 times higher than other authors, presumably because of the very high inoculum titre used, of 10^9 organisms/ml rather than the more usual 10^4-10^5.

Discussion and Conclusions

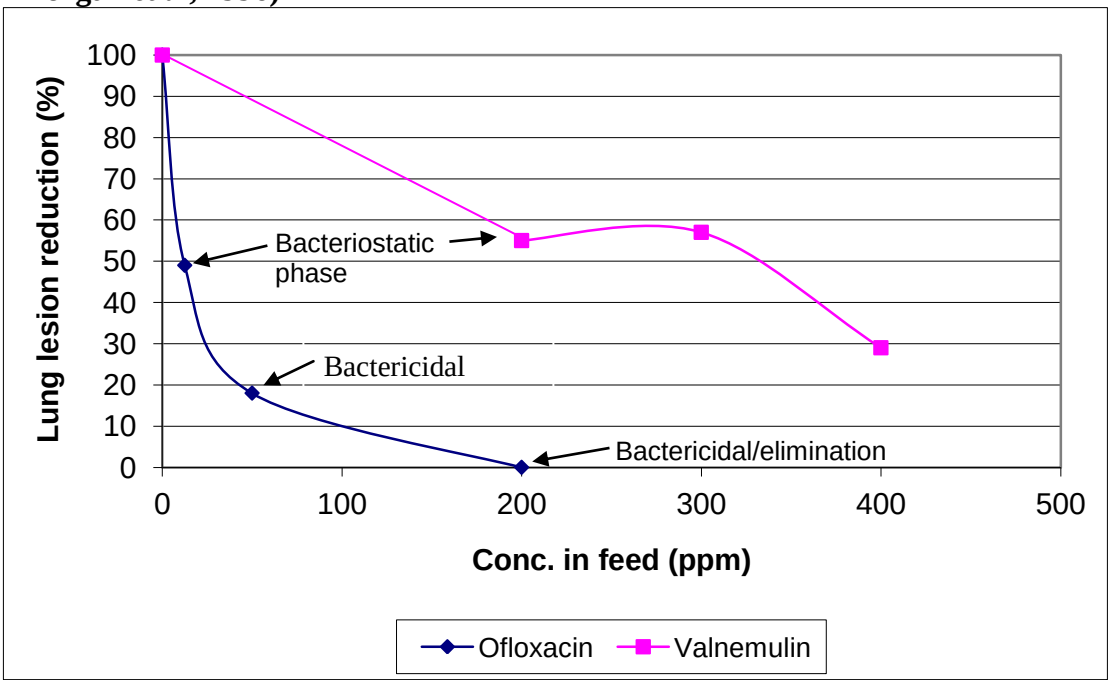
Enzootic pneumonia is a complex disease. It is also difficult to draw conclusions regarding PK/PD relationships from the available PK data, MIC data and trial results. *M. hyopneumoniae* is both difficult and slow to grow *in vitro* and the MIC results may vary from investigator to investigator. The choice of broth or agar plates may have an impact, as well as inoculum size, duration of incubation, end point used and drug used. Some drugs are pH sensitive and others are unstable in broth on incubation over several days e.g. chlortetracycline. In some cases, e.g. after prolonged incubation with tylosin, the MIC continues to drift upwards until it reaches the minimum mycoplasmacidal concentration (MMC = MBC) (Goodwin, 1979). There is no CLSI standard methodology at the moment.

Infection challenge models have also varied substantially, not so much by route of administration of the infection, but by timing of treatment. Prevention studies are relatively straightforward in that treatment commences around the time of infection. However, observation periods after treatment are very variable. As lesions can take over four weeks to form, the timing of treatment studies is more complex. Should they start before four weeks after infection, during the lesion development stage or when the lesions have become well-established, but then they may be naturally regressing after another 3-4 weeks of treatment and observation? Studies have not been standardised. There is an obvious overlap in timing between many of the studies but, in effect, this is

what is happening in a field infection, where there are several stages of lesion development at any one time, depending on when the pig succumbs to the disease.

The effects of the drugs can be divided into three categories, bacteriostatic, bactericidal and elimination of the organism and can be alternatively subdivided into prevention and treatment. The fluoroquinolones are considered bactericidal antimicrobial drugs. Their bacteriostatic phase may be quite narrow in dosage terms before they become bactericidal and elimination is possible, as demonstrated in the case of another fluoroquinolone from Japan, ofloxacin. In the case of valnemulin, a pleuromutilin, it is primarily considered as a bacteriostatic antibiotic, so that its bacteriostatic phase should be much wider (see Figure 2.2). In a prevention study it is unlikely that immunity to *M. hyopneumoniae* will have much of an effect in the first four weeks, hence the lesions continue to develop after cessation of treatment unless the organism is killed.

Figure 2.2. Comparative effect of ofloxacin (bactericidal) and valnemulin (bacteriostatic) in the prevention of enzootic pneumonia (*Kuwano *et al*, 1992; *Morgan *et al*, 1996)



It is thought likely that primarily bacteriostatic antimicrobials, such as tetracyclines, pleuromutilins, macrolides and lincosamides would attain bactericidal levels less readily and especially a mycoplasma elimination level, as very high inclusion levels would be required. It would appear that the majority of these drugs give an intermediate (bacteriostatic) effect, although chlortetracycline appears to be highly effective in preventing lesions developing (95%), but not nearly as effective for treatment (36%), confirming its bacteriostatic effect and a more bactericidal effect would be required. Elimination has been reported with these medications (*Burch and Woolfenden, 2010) but only in fully immune adult animals in herds, which have been partially separated from young growing stock.

Many of the treatment studies are almost late prevention in character, starting under four weeks post-infection. Good responses were achieved with the fluoroquinolone, enrofloxacin (92%), in a mixed infection (*Simon *et al*, 1990), but this was reduced after an observation period of 14 days, to 79%, suggesting that it was having a bactericidal effect, but had not totally eliminated the infection, which therefore continued to develop. Once treatment has been established, consolidated lesions may alter some of the pharmacokinetic parameters, e.g. concentration in the lesion. It is interesting that the responses to a combination of pleuromutilins and chlortetracycline (tiamulin + chlortetracycline 76%; valnemulin + chlortetracycline 86-93%) gave improvements over chlortetracycline alone (36%). Burch *et al* (1986) reported a clinical superiority in the field with tiamulin and chlortetracycline over chlortetracycline alone and *Kitadai *et al* (1998) demonstrated an increased bactericidal effect when valnemulin and chlortetracycline were combined in a killing curve study with *P. multocida*, which might explain this phenomenon.

Relating pharmacokinetic parameters to clinical effect is not straightforward. There has been debate over the significance of antimicrobial lung levels in comparison with blood levels. *M. hyopneumoniae* live primarily on the cell surface and are especially concentrated on the bronchiolar epithelium. Thus the relevance of lung concentrations, which presumably are mainly intracellular in the alveolar cells, is unclear and non-protein bound concentrations in the blood or extracellular fluids are probably more significant. Mouton *et al* (2008) went as far as saying that the use of concentrations of drugs in tissue homogenates is unjustifiable since most bacterial infections are located in the extracellular compartment. Determining concentrations of some antimicrobials at the site of infection is also quite difficult because they are below the limit of quantification or detection in some cases. However, they may still be above the MICs for *M. hyopneumoniae* e.g. valnemulin's MIC90 is 0.0049µg/ml and tylvalosin's is <0.0125µg/ml. The limit of detection in plasma or tissues is of the order of 0.02-0.05µg/ml. The **lung concentration** however may act as an indirect **surrogate marker** for some drugs for what is happening at different sites and has the advantage of magnifying the antimicrobial concentration, usually by positive diffusion gradients, to a detectable limit. Sometimes the lung/plasma relationship data is available. For example, enrofloxacin's lung/serum ratio is about 2.7 (Anon, Baytril premix product literature) and tiamulin's is about 19 (*Forster *et al*, 1982) in the pig. In theory, then the AUC/MIC lung could be divided by the lung/serum ratio to give you the AUC/MIC for plasma/serum and the MMC/MIC ratio to give a comparable AUC/MIC serum figure from which one can predict from the PK/PD integration figures, what effect the antimicrobial is having, whether bacteriostatic, bactericidal or even potentially eliminatory.

Calculations and example for tiamulin (DB)

Formula: AUC 24h lung / (Lung: serum ratio – **19: 1**) / MIC (**0.025µg/ml**) / (MMC: MIC ratio – **5: 1**) = AUC/MMC value h

Using tiamulin 50mg/kg (Goodwin, 1979) 1000ppm in feed for **treatment of EP**

217.2 / 19 / 0.025 / 5 = AUC/MMC value **91h** = 69% reduction in lesions for **treatment**, a **bactericidal** effect.

Using tiamulin 10mg/kg (Schuller et al, 1977) 200ppm for **prevention of EP** lesion development

43.4 / 19 / 0.025/ 5 = AUC/MMC **18h** = 58% reduction of lesions for **prevention**, a **bacteriostatic** effect.

More work is required to prove this hypothesis and it is hoped that it will lead to further investigation by researchers in this field and further clarification of the mode of action of bacteriostatic antimicrobials in the prevention and treatment of swine enzootic pneumonia.

2.3 Common bacterial respiratory infections – *Actinobacillus pleuropneumoniae, Pasteurella multocida* and *Haemophilus parasuis*

Introduction

In the previous section, the author (DB) showed that the plasma PK/PD relationships for bactericidal antimicrobial compounds such as enrofloxacin and ofloxacin did achieve a good bacterial kill and lung lesion reduction against *M. hyopneumoniae* (*Simon *et al*, 1990; *Kuwano *et al*, 1992). The basic PK/PD relationship of plasma AUC/MIC worked well when the MIC was similar to the MBC or MMC but when the MBC/MIC ratio is much higher, e.g. for bacteriostatic drugs, such as tetracyclines, macrolides, lincosamides and pleuromutilins, the classic PK/PD relationships no longer applied. However, it could be restored by the use of the MBC in the calculations. Then they were acting more like the bactericidal drugs. Lung concentrations could be used as a surrogate indicator, when plasma concentrations were low and could not be easily measured and then the lung/serum or plasma concentration ratio could be used in the equation.

Some antimicrobials accumulate in high concentrations in lung tissue relative to plasma, e.g. tiamulin, tilmicosin, tulathromycin, whereas others achieve only similar or slightly higher lung levels to plasma, such as the tetracyclines, fluoroquinolones and penicillins but also tylosin, a macrolide. This section explores the potential relationship of antimicrobial concentrations in the plasma, lung and leucocytes to MICs against the common porcine respiratory bacteria, such as *A. pleuropneumoniae, P. multocida* and *H. parasuis* in comparison with *M. hyopneumoniae* and their effect on clinical efficacy and antimicrobial susceptibility patterns. Much has been made of lung and macrophage levels to explain the antimicrobial activity and efficacy of tilmicosin against these bacteria but some authors (Lees *et al*, 2006; Mouton *et al*, 2008; *Toutain, 2008) think the only reliable PK parameter is plasma concentration. This is to be reviewed.

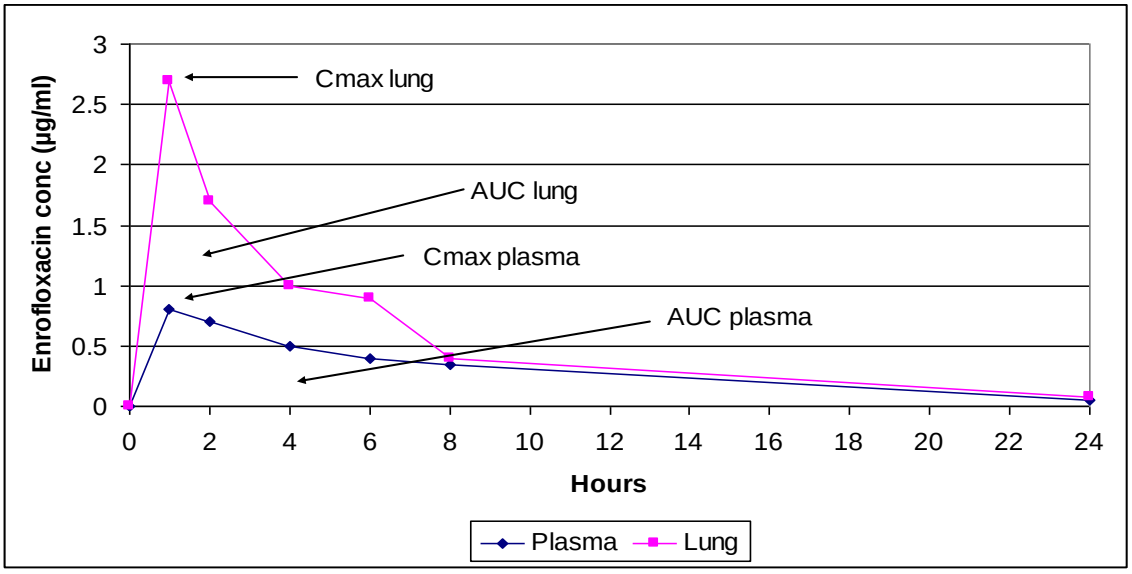
Plasma and lung pharmacokinetics of various antimicrobials

Plasma concentrations differ from but are related to concentrations in the lung. Lung concentration depends on a number of factors such as lipid solubility and cell membrane penetration, as well as dissociation constants (pKa) for acids and bases and subsequent ionisation and possible entrapment in the alveolar cell. Most drugs concentrating in lung tissue are usually weak bases, which become more ionised in slightly higher acid conditions intracellularly (*Keen, 1990). However, plasma and lung concentrations are dynamic and potentially the lung may act as a local reservoir, prolonging activity but may also act as a drain, limiting local plasma and extracellular fluid concentrations. Drug concentrations in leucocytes and especially lung macrophages have also been used to explain the efficacy of some compounds when the MICs exceed plasma levels by large margins, yet clinical trials with bacteria with high MICs are still treated successfully with the drug, which has led to this debate.

Enrofloxacin:

Scheer (1987) reported on the concentrations of enrofloxacin in plasma and lung of pigs after injecting 2.5mg/kg bodyweight (Figure 2.3). A microbiological assay method was used for both plasma and lung tissues. This determines the antimicrobial activity rather than identifying solely a specific substance by high pressure liquid chromatography (HPLC) analysis. This may fail to identify biologically active metabolites, which in the case of enrofloxacin is ciprofloxacin, which is also highly active.

Figure 2.3. Plasma and lung concentrations of enrofloxacin following a single injection at 2.5mg/kg bodyweight in pigs (Scheer, 1987)



The **Cmax lung** for enrofloxacin was **2.7µg/g** and the **Cmax plasma** was **0.8µg/ml** and the **Cmax lung/plasma ratio** was **3.4: 1**. The **AUC lung** was **15.5µg.h/g** and the **AUC plasma** was **7.2µg.h/ml** whilst the **AUC lung/plasma ratio** was **2.2: 1**. In some reports, there is reported only a single lung concentration in relation to plasma. This can be misleading as there is a possible lag effect in reaching a peak lung concentration from

plasma, and there is also a lag from lung back to plasma. Therefore AUCs probably give a more useful lung/plasma relationship in those cases.

Information on serum and lung concentrations of enrofloxacin after oral administration in feed at 150ppm after one and five days was reported in the product data manual (Baytril I.E.R. 2.5% premix – Bayer). **Serum and lung levels** were at **0.17µg/ml** and **0.42µg/g** after one day, rising to **0.3µg/ml** and **0.92µg/g** after 5 days, respectively (**lung plasma ratio range 2.5-3.1**).

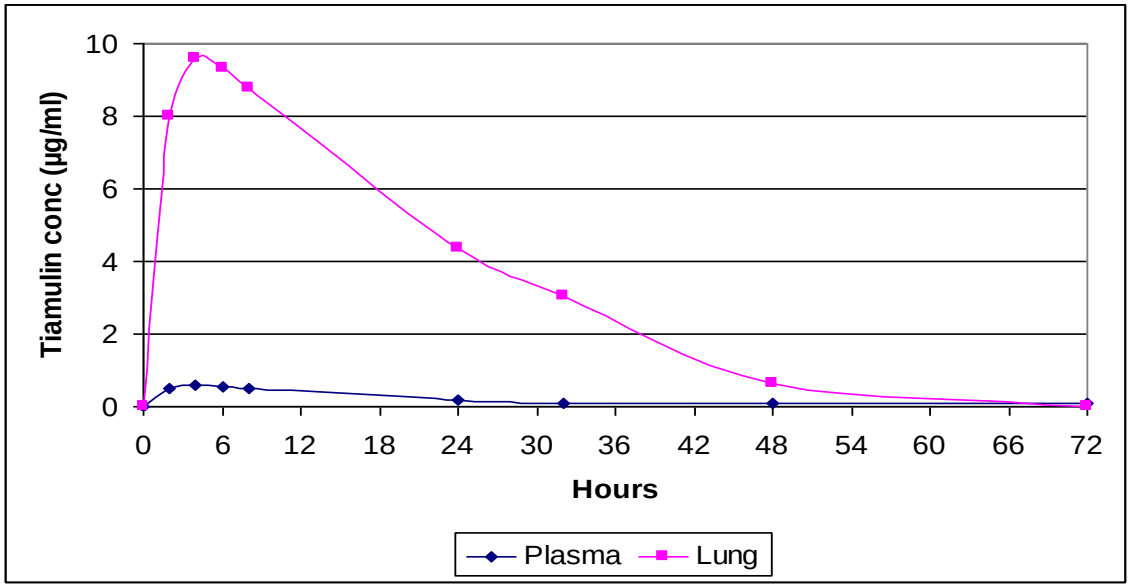
Oxytetracycline:

There are a number of references describing oxytetracycline concentrations in plasma following in feed administration but the most extensive work was by Pijpers *et al* (1991). They measured concentrations of oxytetracycline in plasma following administration in the feed for six days at 400, 800, 1600 and 2400ppm (dosage rate 12.1, 26,4, 54.5, 81.5 and 111.3mg/kg bwt/day respectively), using both HPLC and microbiological methods and relatively similar amounts were found by the two methods. The peak concentrations of oxytetracycline were 0.22, 0.50, 1.43, and 2.14µg/ml respectively. Recovery rates from spiked plasma ranged from 57-72%. Pijpers *et al* (1994) determined plasma and lung concentrations following administration of oxytetracycline in the feed at 400, 800 and 1600ppm (dosage rate 14, 29 and 60mg/kg bwt/day) for six days. The pigs were also infected with *A. pleuropneumoniae,* as part of an artificial challenge study. Slightly higher lung concentrations were obtained than in plasma, but slightly lower plasma concentrations were found than in the first study (see Table 2.11). The **lung/plasma ratio** ranged from **1.09-1.36: 1**.

Tiamulin:

*McKellar *et al* (2004) described the lung/plasma relationships for tiamulin following an injection at a dosage of 15mg/kg. The plasma and lung concentrations were assayed using a microbiological assay, again recording both active substance and microbiologically active metabolites, some of which have 12.5-50% activity in comparison with the parent compound (Lykkeberg *et al*, 2007) (see Table 2.11).

Figure 2.4. Plasma and lung concentrations of tiamulin following a single injection at 15mg/kg bodyweight in pigs (*McKellar *et al*, 2004)



The Cmax for lung was **9.6µg/g**, that for plasma was **0.61µg/ml** and the **Cmax lung/plasma ratio** was **15.7:1**. The AUC lung was **231.5µg.h/g**, that for plasma was **12.8µg.h/ml** but the **AUC lung/plasma ratio** was **18.1:1**, which is a substantially higher ratio than for enrofloxacin and oxytetracycline.

*Anderson *et al* (1994) reported on tiamulin lung concentrations found after feed incorporation at 38.5, 110 and 220ppm, and water medication at 60, 120 and 180ppm (see Table 2.11). Corresponding plasma concentrations were not reported.

*Nielsen and Szancer (1998) reported on the uptake of tiamulin by neutrophils at different concentrations and over time. After 20h, the uptake was between **4.9-18.2** times the extracellular concentrations of 11 to 24µg/ml.

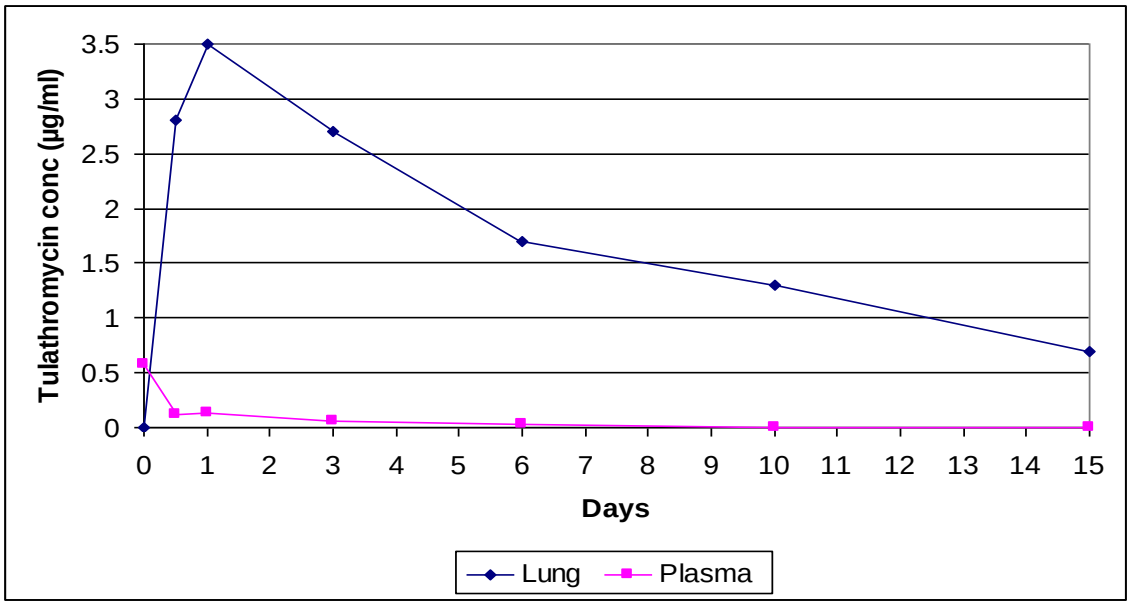
Tilmicosin:

*Thomson et al (1994a & *1994b) described the serum concentration of tilmicosin in relation to lung concentration after feeding at 400ppm for 14 days. Serum and lung concentrations peaked at about 10 days with levels of **0.23µg/ml** and **2.59µg/g**, respectively, by HPLC assay, which gave a **lung/serum concentration ratio** of **11.3:1**. *Blais and Cumberland (1994) demonstrated that tilmicosin accumulated in alveolar macrophages, up to **75 times** following incubation in a solution of 20µg/ml for 24h. *Stoker *et al* (1996) showed that after feeding 400ppm tilmicosin for 14 days average serum concentrations were **0.039µg/ml**, lung concentrations were **1.69µg/g**, tracheal epithelium was **2.19µg/g** and lung macrophages were **7.19µg/ml**. The **lung/serum ratio** was **43:1**, which is substantially higher than the earlier work (*Blais and Cumberland, 1994), presumably due to the lower serum levels recorded and the **macrophage/serum levels** were **184:1**, also much higher.

Tulathromycin:

It was not until the introduction of tulathromycin in 2002, and the lack of correlation of plasma concentration to clinical outcome for the treatment of bacterial (*A. pleuropneumoniae*) respiratory infections that the PK/PD debate over the significance of plasma concentrations and lung concentrations commenced in veterinary medicine.

Figure 2.5. Plasma and lung concentrations of tulathromycin following a single injection at 2.5mg/kg bodyweight in pigs (Benchaoui *et al*, 2004)



The **lung Cmax** for tulathromycin was **3.47µg/g** and the **plasma Cmax** was **0.62µg/ml**. The Cmax lung/plasma ratio was **5.6:1**. The AUC lung (last time point 15 days) was **615µg.h/g** and the AUC plasma was **12.0µg.h/ml**. Therefore, the **AUC lung/plasma ratio** was **51.3:1**, which is substantially higher than enrofloxacin. At 6 days, the AUC lung/plasma ratio was **29:1**. The assays were carried out by LC-MS/MS but the metabolism of tulathromycin is slow and the majority of the drug is excreted unchanged in faeces and urine.

Tulathromycin has been shown to accumulate in neutrophils and alveolar macrophages in pigs at **16.6** and **8.1** times the extracellular fluid concentration (Evans, 2005).

A number of antimicrobial products and their plasma and lung levels are highlighted in Table 2.11. Estimations (E) may also be included based on lung/plasma ratio data, where data is deficient, for example, with tiamulin and tylosin.

Table 2.11. Antimicrobial concentrations in lung and plasma and Cmax lung/plasma (L/P) ratios and AUC lung/plasma (L/P) ratios (Calculations DB)

Drug/Ref	Formulation	Dose (mg/kg)	Lung Cmax	Plasma Cmax	**Cmax L/P ratio**	Lung AUC	Plasma AUC	**AUC L/P ratio**
Enrofloxacin								
Scheer, 1987 (M)	Inj (24h)	2.5	2.7	0.8	**3.4:1**	15.5	7.2	**2.2:1**
Premix brochure data (Anon.)	In feed 150ppm	7.5	0.92	0.3	**3.1:1**	22.1	7.2	**3.1:1**
Ceftiofur								
Brown *et al*, 1999 (H)	Inj Na Cef Inj Cef HCl (72h)	3 3		15.8 11.8			196 216	
Tiamulin								
*McKellar *et al*, 2004 (M)	Inj (72h)	15	9.6	0.61	**15.7:1**	231.5	12.8	**18.1:1**
*Anderson *et al*, 1994 (M)	In water 60ppm 120ppm 180ppm	 6.2 13.2 20.9	 1.1 4.3 8.5	 0.06(E) 0.24(E) 0.47(E)	**Used 18.1:1**			
*Anderson *et al*, 1994 (M)	In feed 110ppm 220ppm	 6.6 13.2	 1.5 2.0	 0.08(E) 0.11(E)				
*Nielsen & Szancer, 1998					**PM/P 4.9-18.2:1**			
Tylosin								
Hoffman *et al*, 1983 (M)	Inj (24h)	10	3.37	3.49	**0.96:1**	21.3	16.1	**1.3:1**
*Ibayashi *et al*, 1994 (M)	In feed 110ppm	5.5	<0.05	<0.04(E)	**Used 1.3:1**			
Tilmicosin								
*Thomson *et al*, 1994a (H)	In feed 200ppm 400ppm	 10 20	 1.43 2.59	 <0.1 0.23	**11.3:1**			
*Stoker *et al*, 1996 (H)	In feed 400ppm	20	1.69 MPs 7.2	0.039	**43:1 MP/P 184:1**			
*Blais & Chamberland, 1994					**MP/P 75:1**			
Tulathromycin								
Benchaoui *et al*, 2004 (H)	Inj LA form 15days	2.5	3.47	0.62	**5.6:1**	615	12.0	**51.3:1**

Drug/Ref	Formulation	Dose (mg/kg)	Lung Cmax	Plasma Cmax	**Cmax L/P ratio**	Lung AUC	Plasma AUC	**AUC L/P ratio**
Evans, 2005					**PM/P 16.6:1 MP/P 8.1:1**			
Lincomycin								
*Swenson & Barbiers, 1976 (M)	Inj	11	12.5	7.03	**1.8:1**			
*DeGeeter *et al*, 1980 (M?)	In feed 110ppm 220ppm	 5.5 11	 0.66 1.13	 0.16 0.14	 **4.1:1** **8.1:1**			
Oxytetracycline								
Banting & Baggot, 1996 (M)	Inj LA form (48h)	20		4.68			86.6	
Asanuma *et al*, 1986 (M)	In feed 400ppm	 20	 0.15	 0.11	 **1.4:1**	 2.36	 2.0	 **1.2:1**
Pijpers *et al*, 1994 (H)	In feed 400ppm 800ppm 1600ppm	 20 40 80	 0.23 0.42 0.78	 0.25 0.57 0.83	 **1.09:1** **1.36:1** **1.06:1**			
Chlortetracycline								
*Jacobson *et al*, 1994 (M)	In feed 1000ppm	 50	 0.56	 0.44	 **1.3:1**			
Asanuma *et al*, 1986 (M)	In feed 400ppm	 20	 0.66	 0.35	 **1.9:1**	 11.75	 5.78	 **2:1**

Key: Inj = injection; M = microbiological assay; H = HPLC assay; E = Estimate; MPs = macrophages; PMs = polymorphs; P – plasma; L = lung

In contrast with a parenteral dosing regimen, when an antimicrobial is administered orally in feed or drinking water over a 24h period, the plasma concentrations and resulting lung concentrations are lower and less undulating. The Cmax is usually lower, but the AUC for a given dose may be similar or lower depending on several factors, such as absorption from the gut, metabolism in the liver and also production of bioactive metabolites. Food usually slows the absorption and in the case of the tetracyclines calcium may bind to the drug. However, the absorption may or may not reduce the overall bioavailability or the AUC 24h unless the metabolism is mainly in the liver.

With bacteriostatic antimicrobial drugs the time the concentration exceeds the MIC is the important correlation for efficacy, assuming the MIC is an in-vitro parameter that applies. Therefore AUC divided by 24h gives the equivalent of a steady state effect for calculation purposes. For penicillins (principally time dependent) an AUC of 100-120 / 24h = **4.2-5.0** and four times the MIC is often a 'rule of thumb' level to achieve a good clinical or bactericidal effect. With bacteriostatic drugs, an AUC of 24 can be considered

inhibitory (1 times MIC over a 24 hour period) but a cidal or even eliminatory activity would be dependent on the MBC/MIC ratio, which varies for organism and antimicrobial drug and can be several times higher than one. Concentration of drugs in macrophages and their activity will also be discussed.

Pharmacodynamics of various antimicrobial drugs on respiratory associated bacteria

Classically, the MIC of the antimicrobial against the organism is the important measurement of activity and potential efficacy. For several isolates (ideally more than 10), then the MIC 50% for a population, and MIC 90% and range, can be determined. This gives a broad indication of the susceptibility of the population, but it should be put in context of what are achievable antimicrobial concentrations, say in plasma or lung or other target tissue, possibly leucocytes and fluids.

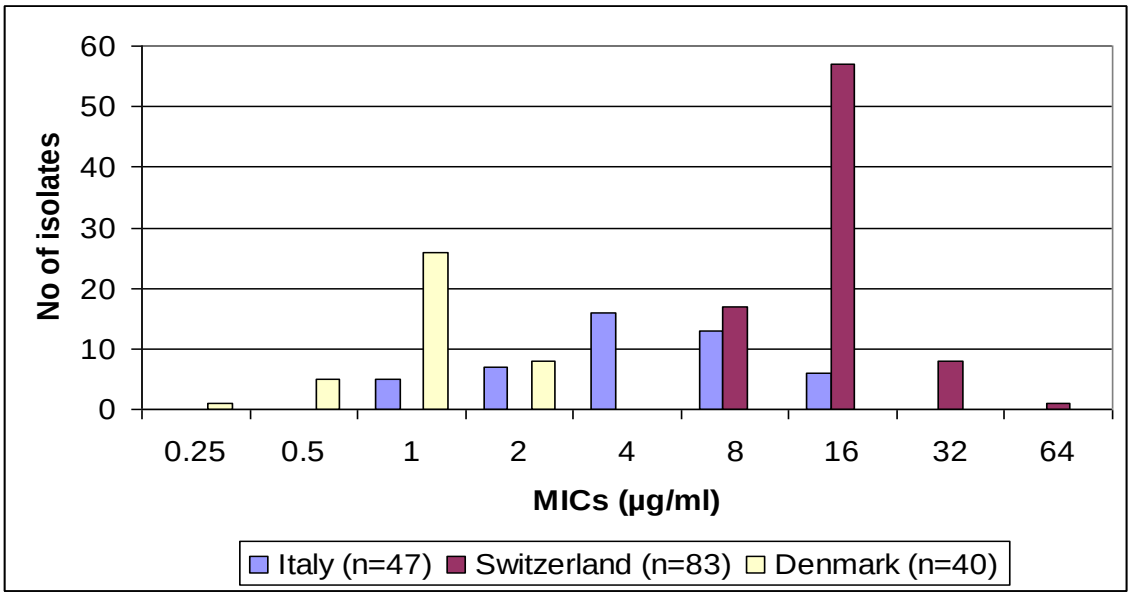
A. pleuropneumoniae:

Table 2.12. MICs of antimicrobial drugs against *A. pleuropneumoniae*

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Aarestrup and Jensen, 1999 – Denmark – 40 isolates (chocolate agar)			
Ceftiofur	≤0.03	≤0.03	≤0.03
Enrofloxacin	≤0.03	≤0.03	≤0.03
Tiamulin	4.0	4.0	0.5-4.0
Tylosin	8.0	16	4.0-16
**Casals et al, 1990 – Denmark – 26 isolates (Danish blood agar)*			
Tiamulin	4.0	4.0	1.0-8.0
Chang et al, 2002 – Taiwan – 60 isolates (Veterinary fastidious agar – NCCLS))			
Ceftiofur	0.03	0.03	0.03-0.12
Enrofloxacin	0.5	8.0	0.03-16
Lincomycin	16	32	4.0-64
Tetracycline	8	16	0.25-64
Matter et al, 2007 – Switzerland – 83 isolates (Veterinary fastidious medium - NCCLS)			
Ceftiofur	≤0.5	≤0.5	≤0.5
Enrofloxacin	0.03	0.03	0.03-1.0
Tiamulin	16	32	8-64
Tilmicosin	16	16	8-64
Erythromycin	8.0	8.0	4.0-8.0
Tetracycline	0.5	0.5	0.5-32

Consistently, there is a high level of susceptibility to ceftiofur and also enrofloxacin in most reports; although in Taiwan there is some degree of resistance development to enrofloxacin. The reported MICs for tiamulin are particularly variable and this is likely to be due to different culture methods, e.g. media, inoculum density and pH (*Casals *et al*, 1990; Aarestrup and Jensen, 1999; Sidoli *et al* 1984; Matter *et al*, 2007) and seems to be more associated with fastidious growing organisms like *A. pleuropneumoniae* (Figure 2.6). There were no apparent resistance patterns developing.

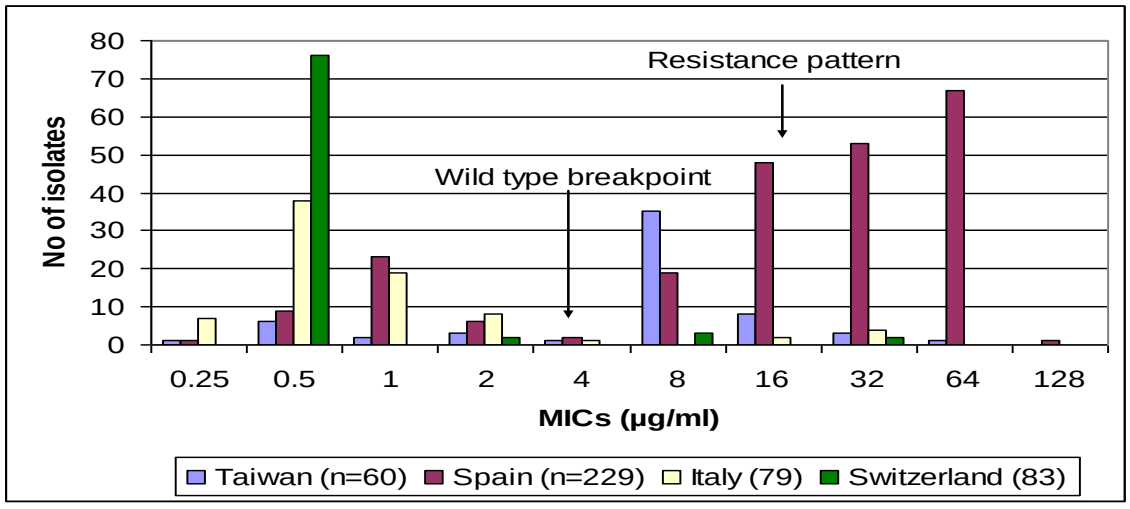
Figure 2.6. Comparison of MIC results for tiamulin against *A. pleuropneumoniae* (Sidoli *et al,* 1984(It); Matter *et al*, 2007 (Ch); Aarestrup and Jensen, 1999 (Dk)



Evans (2005) and Godinho *et al* (2005) also described a wide variation in MIC findings with tulathromycin, with MICs varying from 32µg/ml down to 0.25µg/ml depending on the pH, presence of CO2 and serum in the media.

The susceptibility to tetracycline is also very variable. High levels of resistance have been reported in Taiwan (Chang *et al*, 2002) and Spain (Guttierrez –Martin *et al*, 2006), probably associated with high levels of tetracycline use, in comparison with Switzerland (Matter *et al*, 2007) and in Italy (Sidoli *et al*, 1984) where the data is from nearly 20 years previously.

Figure 2.7. Comparison of susceptibility patterns of tetracycline against *A. pleuropneumoniae* from various countries (Chang *et al,* 2002; Guttierrez-Martin *et al,* 2006; Sidoli *et al,* 1984; Matter *et al,* 2007)



Tylosin has high MICs, especially in comparison with the plasma and lung concentrations achieved with clinical dose rates, and similarly lincomycin. Tilmicosin has reportedly high MICs, but there is limited comparative MIC information available. Erythromycin has MICs of approximately half those of tilmicosin (Shryock *et al*, 2002) and is more commonly reported than tilmicosin.

Pasteurella multocida:

Table 2.13. MICs of antimicrobial drugs against *P. multocida*

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Vera-Lizarazo et al, 2006 – Spain – 63 isolates ***(1987-1988)***			
Ceftiofur	≤0.5	≤0.5	≤0.5
Enrofloxacin	≤0.12	≤0.12	≤0.12
Oxytetracycline	1.0	1.0	≤0.25-16
Chlortetracycline	0.5	1.0	≤0.5-16
Tiamulin	32	32	≤4.0-64
Tylosin	10	20	NR
Tilmicosin	≤4	≤4	≤4.0-64
Vera-Lizarazo et al, 2006 – Spain – 132 isolates ***(2003-2004)***			
Ceftiofur	≤0.5	≤0.5	≤0.5-1.0
Enrofloxacin	≤0.12	≤0.12	≤0.12
Oxytetracycline	2.0	8.0	0.5-16
Chlortetracycline	2.0	8.0	≤0.5-16
Tiamulin	16	32	≤4.0-64
Tylosin	10	20	NR
Tilmicosin	≤4.0	8.0	≤4.0-16

There have been relatively minor changes to the susceptibility of *P. multocida* over the last 14 years in Spain except to the tetracyclines, where there is evidence of resistance emerging. Based on the concentrations achieved after parenteral dosing, many isolates could be susceptible, but based on plasma concentrations achieved following in-feed medication, there is potentially a high level of resistance to the tetracyclines (>10%). Tiamulin MIC 50s have decreased in the same period, but are still very high.

Haemophilus parasuis:

Table 2.14. MICs of antimicrobial drugs against *H. parasuis*

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Aarestrup et al, 2004 – Denmark – 52 isolates (Veterinary fastidious medium, (VFS))			
Ceftiofur	0.03	0.03	0.03
Ciprofloxacin	0.015	0.06	0.015-0.5
Tetracycline	1.0	2.0	0.06-2.0
Tiamulin	4.0	8.0	1.0-16
Tilmicosin	2.0	2.0	2.0-4.0
Martin-de la Fuente et al, 2007 – ***United Kingdom*** *– 30 isolates (VFS)*			

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Ceftiofur	≤0.5	1.0	≤0.5-2.0
Enrofloxacin	≤0.12	0.25	≤0.12-1.0
Oxytetracycline	0.5	4.0	0.25-16
Tiamulin	≤4.0	16	≤4.0-32
Tilmicosin	≤4.0	8	≤4.0-8.0
*Martin-de la Fuente et al, 2007 – **Spain** – 30 isolates (VFS)*			
Ceftiofur	≤0.5	4.0	≤0.5-16
Enrofloxacin	0.25	4.0	≤0.12-4.0
Oxytetracycline	2.0	16	0.25-16
Tiamulin	≤4.0	64	≤4.0-64
Tilmicosin	16	64	≤4.0-64

The Danish MIC levels were lower than the UK's but Spain's appeared to be quite high in comparison with the UK. The MIC 90s for ceftiofur, enrofloxacin, oxytetracycline tiamulin and tilmicosin were all higher in the Spanish isolates.

Mycoplasma hyopneumoniae:

Table 2.15. MICs of antimicrobial drugs against *M. hyopneumoniae*

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Inamoto et al, 1994 – 40 isolates - Japan			
Chlortetracycline	3.1	>100	0.2-≥100
Oxytetracycline	0.2	3.13	0.025-12.5
Lincomycin	0.025	0.1	≤0.0125-0.39
Tiamulin	≤0.0125	0.025	≤0.0125-0.05
Tilmicosin	0.2	0.39	≤0.0125-0.78
Tylosin	0.025	0.1	≤0.0125-0.2
Vicca et al, 2004 – 21 isolates - Belgium			
Enrofloxacin	0.03	0.5	0.015->1.0
Doxycycline	0.12	0.5	0.03-1.0
Oxytetracycline	0.12	1.0	0.03-2.0
Lincomycin	≤0.06	≤0.06	≤0.06->8.0
Tiamulin	≤0.015	0.12	≤0.015-0.12
Tilmicosin	0.25	0.5	≤0.25->16
Tylosin	0.03	0.06	≤0.015->1.0

There were some increases of MIC with time for some of the antimicrobials and resistance to tylosin, tilmicosin and lincomycin was reported in Belgium, but less than10%. By comparison, the MICs for tiamulin, tylosin, tilmicosin and lincomycin against *M. hyopneumoniae* were much lower than against the respiratory bacteria *A. pleuropneumonia, P. multocida* and *H. parasuis.*

Clinical efficacy of antimicrobial drugs against bacterial respiratory infections

To make correlations of PK/PD relationships with clinical effect, it is important to establish the MICs of the organism used in the challenge study. Unfortunately, the MIC data are not always known. Table 2.16 highlights the main indications of various antimicrobials.

Table 2.16. Principal clinical indications of various antimicrobials in the UK (Anon., 2007b)

Name / form	Dose rate (mg/kg)	*M. hyopneumoniae*	Bacteria
Enrofloxacin			
Injection	2.5 / for 3 days	Yes	App, Pm, Bb
Feed 150ppm (not now registered)	7.5 / for 5 days		
Ceftiofur			
Injection	3 / for 3 days	No	App, Pm, Ss
Tiamulin			
Injection	15 / for 3 days	Yes	Sensitive orgs
Feed	1.5-2 / up to 2 months 10 / 10days (not UK)	Yes	-
Water	12-18 / 5 days (not UK)	Yes	Sensitive orgs
Tylosin			
Injection	2-10 / daily	Yes	Sensitive orgs
Water	25 / for 5 days	Yes	-
Feed 100ppm	3-6 / for 21days	Yes	-
Tilmicosin			
Feed 200-400ppm	8-16 / for 15 days	Yes	App, Pm, Hps
Tulathromycin			
Injection	2.5 / single	Yes	App, Pm, Hps
Lincomycin			
Injection	4.5-11 / for 3 days	Yes	Sensitive G+ orgs
Feed 220ppm	11 / for 21 days	Yes	-
Oxytetracycline			
Injection	10 / day	Sensitive orgs	Pm, App, Bb
Injection (LA)	20 / single	Sensitive orgs	Pm + sensitive orgs
Water	10-30 / for 3-5 days	Sensitive orgs	Sensitive orgs
Feed 400-1000ppm	20 / for 15 days	Sensitive orgs	Sensitive orgs
Chlortetracycline			
Water	20 / for 5 days	Yes	Pm, Ss, Bb
Feed 300ppm	10-20 / for 5-7 days	Sensitive orgs	Sensitive orgs

App = *A. pleuropneumoniae*; Pm = *P. multocida*; Bp = *B. Bronchiseptica*; Ss = *Streptococcus suis*; Hps = *H. parasuis*; G+ = Gram positive; Sensitive organisms = organisms are not specified in older registrations

Enrofloxacin:

Enrofloxacin, administered in feed, was tested against a challenge isolate of *A. pleuropneumoniae* by Smith *et al* (1991). Pigs were given enrofloxacin at 0, 32 and 150ppm and then infected with the challenge strain 4 hours later. Dosing was for seven days, and then the pigs were necropsied. The pneumonic lung lesions scores were reduced by 72% and 88% in the enrofloxacin 32 and 150ppm treated groups, respectively, in comparison with the untreated controls (see Table 2.17). No *A. pleuropneumoniae* were isolated from the enrofloxacin 150ppm-treated group but from 17% of the 32ppm group and 92% of the untreated controls. The **MIC of the organism** was given as >0.01 to <0.05µg/ml and **0.03µg/ml** was used in the calculations.

Table 2.17. Plasma and lung PK/PD relationships of enrofloxacin for the prevention of *A. pleuropneumoniae* (Calculations DB)

Treatment Enrofloxacin (ppm)	Lung lesion scores (%)	MIC (µg/ml)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC (h)	AUC lung / MIC (h)
0	100	>0.01-<0.05	0			
32	28 (-72%)	0.03 (used)	2(E)	6.1(E)	48(E)	147(E)
150	12 (-88%)	0.03 (used)	**10**	31	**240**	736

E = estimated

The best clinical response was achieved in the 150ppm enrofloxacin group and the control of infection was very good, eliminating the challenge organism. Surprisingly, the 32ppm level was also quite effective in preventing the development of lung lesions in the majority of pigs i.e. a bacteriostatic effect. The Cmax plasma/MIC was **10** and the AUC plasma/MIC was **240h** at 150ppm, which was approximately what would be expected ratios for a fluoroquinolone.

Oxytetracycline:

Oxytetracycline was used in a number of dose-titration studies (Pijpers *et al*, 1994). Pigs were given 0, 400, 800 and 1600ppm oxytetracycline **prophylactically** in feed and challenged with an isolate of *A. pleuropneumoniae* with an **MIC of 1.0µg/ml**. The percentages of pigs with pneumonia were 100%, 67%, 27% and 0%, respectively.

Table 2.18. Plasma and lung PK/PD relationships of oxytetracycline for the prevention of *A. pleuropneumoniae*

Treatment Oxytetracycline (ppm)	Pigs with App lesions	MIC (µg/ml)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC (h)	AUC lung / MIC (h)
0	100	1.0	0	0	0	0
400	67	1.0	0.23	0.25	5.5	6.0
800	27	1.0	0.42	0.57	10.1	13.7
1600	0	1.0	0.78	0.83	**18.7**	**19.9**

The MIC of the isolate ranged from >0.5 to 1.0µg/ml because of doubling dilutions, so the figure of AUC/MIC is approaching 24 (**18.7h and 19.9h**) for both plasma and lung, suggesting that there is little difference between the two and the 1600ppm of oxytetracycline is giving a good, bacteriostatic protection from the challenge infection, as judged by lung lesion reduction. Pijpers *et al* (1991) showed in an earlier PK study that the recovery of oxytetracycline was between 57-72%, using their HPLC method in comparison with spiked samples, so the final plasma calculations may be underestimated.

Tiamulin:

Tiamulin has been used in artificial challenge studies with *M. hyopneumoniae* and also dose titration studies with *A. pleuropneumoniae*, where the MICs for the challenge organisms were determined.

Hannan *et al* (1982) showed that tiamulin caused a marked reduction in lung lesions when given to piglets, which had been infected with a lung homogenate containing *M. hyopneumoniae* with an MIC of 0.1µg/ml. Piglets were artificially reared on evaporated milk and infected at about 1 week of age. They were treated two weeks later with tiamulin at 10mg/kg given twice a day for 10 days and necropsied 14 days after treatment. Lung lesions in the control group were scored on average **24.5** based on an average score of 5/lobe (max score 35) and in the tiamulin-treated group **0.56**, a **98% reduction**. *M. hyopneumoniae* was not isolated from the treated pigs but from all five of the untreated controls. A good bactericidal effect was observed. For the PK calculations the lung and plasma concentrations following tiamulin administration at 180ppm were used, as they were similar in dosage terms.

Table 2.19. Comparison of plasma and lung PK/PD relationships of tiamulin for the treatment of *M. hyopneumoniae* (Hannan *et al*, 1982) (Calculations DB)

Treatment	Average lung score	MIC (µg/ml)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC (h)	AUC lung / MIC (h)
Negative control	24.5					
Tiamulin 20mg/kg/day for 10days	0.56 (-98%)	0.1	**4.7**	**85**	**113**	**2040**

The Cmax and AUC plasma/MIC relationship calculations correlate with high clinical efficacy, whereas the lung Cmax and AUC concentrations/MIC give very high figures, suggesting a lack of normal correlation.

*Burch and Klein (2008) reported on a dose-titration study with tiamulin in the drinking water using an *A. pleuropneumoniae* type 5 isolate with an MIC of 4.0µg/ml. Pigs were infected intranasally and when signs of disease started to occur they were allocated to

one of four tiamulin drinking water treatments at 0, 60, 120 and 180ppm for 5 days. The pigs were necropsied 21 days after infection and their lung lesions scored and cultured for *A. pleuropneumoniae*.

Table 2.20. Dose titration study with tiamulin administered in the drinking water for the treatment of *A. pleuropneumoniae* (MIC of 4.0µg/ml) (*Burch and Klein, 2008)

Treatment tiamulin (ppm)	MIC (µg/ml)	Mortality (24h)	Average lung lesion score (%)	Average lung lesions score of surviving pigs	*A. pleuropneumoniae* re-isolation
0	4.0	2/8	100	100	7/8
60		1/8	100	92	6/8
120		1/8	52	19	1/8
180		0/8	2	2	0/8

Table 2.21. Comparison of plasma and lung PK/PD relationships of tiamulin for the treatment of *A. pleuropneumoniae* (Calculations DB)

Treatment tiamulin (ppm)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC	AUC lung / MIC
60 (minor effect)	0.015	0.28	0.36	6.6
120 (inhibitory)	0.06	1.08	1.44	**25.8**
180 (bactericidal / eliminatory)	0.118	2.13	2.82	**51**

From the calculations, it appears that the lung Cmax and AUC / MIC relationships are likely to be more important in comparison with the plasma Cmax and AUC/MIC for tiamulin and respiratory bacteria. Interestingly, the AUC lung/MIC of **25.8h** correlated well with an inhibitory effect and that the AUC lung/MIC of **51h** correlated with a marked bactericidal, even eliminatory effect. These correlations assume that the MIC is representative, however, and suggest a different PK/PD relationship exists, other than plasma concentration and MIC.

Tilmicosin:

Although there have been reported a number of successful artificial challenge models (Moore *et al*, 1996; Paradis *et al*, 2004; Wilson, 2004; Nerland *et al*, 2005), no MIC data for the challenge strains of *A. pleuropneumoniae* were available. Shryock *et al* (2002) reported that there was good clinical efficacy with isolates with MICs up to 16µg/ml, hence this figure was used as the clinical breakpoint but this could not be correlated to

drug concentrations of tilmicosin in plasma, lung or macrophages (*Thomson *et al*, 1994a).

Table 2.22. Dose-titration data for of tilmicosin administered in feed for the prevention of transmission of *A. pleuropneumoniae* from infected seeder pigs (Moore *et al,* 1996)

Tilmicosin level (ppm) from day -7 to 14	Average lung score (%)	*A. pleuropneumoniae* recovery (%) day 14	ADG (g) day 0-14
0	100^{a}	35^{a}	0.41^{a}
100	65^{b}	13^{b}	0.63^{b}
200	21^{c}	8^{b}	0.67^{c}
300	10^{c}	0^{b}	0.71^{c}
400	8^{c}	0^{b}	0.69^{c}

Key: different letter superscripts significantly different from each other $p = \leq 0.05$

Tulathromycin:

McKelvie *et al* (2005) described the use of enrofloxacin as a positive control in a *M. hyopneumoniae* challenge treatment study testing tulathromycin. Enrofloxacin was given at 5mg/kg (twice recommended dose) for 3 days and tulathromycin was given at 2.5mg/kg as a single dose 5-6 days after a double challenge with *M. hyopneumoniae*. They were slaughtered 12 days later and the lungs examined and scored for enzootic pneumonia lesions. The MIC for tulathromycin was **0.05µg/ml** but the MIC was not reported for enrofloxacin.

Table 2.23. Comparison of tulathromycin PK/PD parameters and enrofloxacin for the treatment of enzootic pneumonia (McKelvie *et al*, 2005) (Analysis DB)

Treatment	Lung lesion score (%)	MIC (µg/ml)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC (h)	AUC lung / MIC (h)
Untreated control	17.2					
Enrofloxacin	1.7 (-90)	-				
Tulathromycin	8.8 (-49)	0.05	**12.4**	69.4	**240**	12,300

The Cmax/MIC for plasma was **12.4** and the AUC plasma/MIC was **240h** for tulathromycin against the *M. hyopneumoniae* isolate used. The resulting lung lesion reduction of **49%** is characteristic of an inhibitory effect against the organism for this type of antimicrobial. No MBC/MIC ratio is presented for tulathromycin against *M. hyopneumoniae*, but a bactericidal effect at 4 times MIC was reported by Evans (2005) against *A. pleuropneumoniae*. By contrast the lung Cmax and AUC/MIC were exceptionally high, suggesting that these concentrations were not applicable. Enrofloxacin was used at double the normal dose and achieved a stronger mycoplasmacidal effect reducing lung lesions by 90%. A predicted MIC of ≤0.12µg/ml, which is within normal MIC limits, could be estimated.

Hart *et al* (2006) tested tulathromycin at 2.5 and 5.0mg/kg as a single injection in pigs in comparison with ceftiofur Na at 3mg/kg given for three consecutive days as a positive control, against a naturally induced contact-challenge infection with *A. pleuropneumoniae* type 1. As signs of clinical disease developed in the in-contact pigs, they were treated (day 0) and monitored for 10 days when they were euthanized and the lung lesions scored. The MICs of tulathromycin and ceftiofur Na against the organism were 16 and 0.063µg/ml, respectively.

Table 2.24. Comparative results of tulathromycin and ceftiofur Na for the treatment of *A. pleuropneumoniae* (App) (Hart *et al*, 2006)

Treatment	Deaths (%)	Lung lesions (%)	Weight gain (kg) (Day 0-10)	App re-isolated (%) (Day 10)
Untreated control	12	29.1	1.42	68
Tulathromycin 2.5mg/kg	4	10.1	4.23	64
Tulathromycin 5mg/kg	0	7.9	5.05	36
Ceftiofur Na 3mg/kg 3 days	0	10.0	4.52	56

Table 2.25. Comparison of tulathromycin PK/PD parameters and ceftiofur Na for the treatment of *A. pleuropneumoniae* (Calculations DB)

Treatment	MIC (µg/ml)	Cmax plasma / MIC	Cmax lung / MIC	AUC plasma / MIC (h)	AUC lung / MIC (h)
Tulathromycin 2.5mg/kg	16	0.039	0.22	0.75	38.4
Tulathromycin 5mg/kg	16	0.078	0.43	1.5	**76.8**
Ceftiofur Na 3mg/kg 3 days	0.063	**251**	-	**3111**	-

The PK/PD results are very contrasting. For tulathromycin, there would appear to be a dose-related inhibitory effect, but it is more related to AUC lung/MIC not AUC plasma or Cmax plasma. The authors pointed out that the MIC of 16µg/ml was possibly overestimated (by four dilutions) due to culture method. In addition, the drug might concentrate in macrophages, like tilmicosin. Thus, the MIC would be nearer 1.0µg/ml and the AUC plasma/MIC at a dose of 5mg/kg bwt would be **24h** and AUC lung/MIC would be **1229h**. *Illambas *et al* (2008) showed a 50 fold reduction in tulathromycin MICs, when bacteria were cultured in serum in comparison with broth. This would increase the AUC plasma/MIC to **75h** and AUC lung/MIC to **3840h**. If this were divided by 10days duration of the study the AUC plasma/MIC would be 7.5h, which is very low and the AUC lung/MIC would be 384h, which is about the expected PK/PD integration value. By comparison, ceftiofur Na provided a very strong inhibitory effect, especially in the first few days, which deteriorated subsequently. The Cmax/MIC and

AUC/MIC ratios were substantially greater than the figures of 12 and 120h, respectively, associated with a bactericidal effect. The clinical effect was very good by day 4 (zero score) but by day 10, 36% of the pigs were showing clinical signs, even higher than the untreated controls (16%), while both the tulathromycin groups were stable at 8 and 4%, respectively, for the 2.5 and 5mg/kg dose rates.

These examples show why lung homogenate concentrations may be considered an important factor in assessing the antibacterial effect of a drug, especially when the drugs, such as tiamulin, tilmicosin and tulathromycin, concentrate in lung tissue. High drug lung concentrations offer an attractive and simple explanation of efficacy. An organism such as *A. pleuropneumoniae* can cause an acute degree of necrosis in lung tissue, which may also have a contributory factor. By contrast, *M. hyopneumoniae* is mainly surface dwelling and causes a comparatively mild and chronic infection and plasma/MIC concentrations correlate well with efficacy. However, where antimicrobials do not concentrate in the lung to any marked degree, such as enrofloxacin, ceftiofur and oxytetracycline, plasma/MIC levels appear to be quite satisfactory in relating PK/PD relationships and their efficacy. There have been reported problems determining the MICs of the more fastidious respiratory bacteria, such as *A. pleuropneumoniae* (Aarestrup and Jensen, 1999; Godinho *et al*, 2005), which suggests that the problem of PK/PD integration may have arisen from the PD component of these drugs.

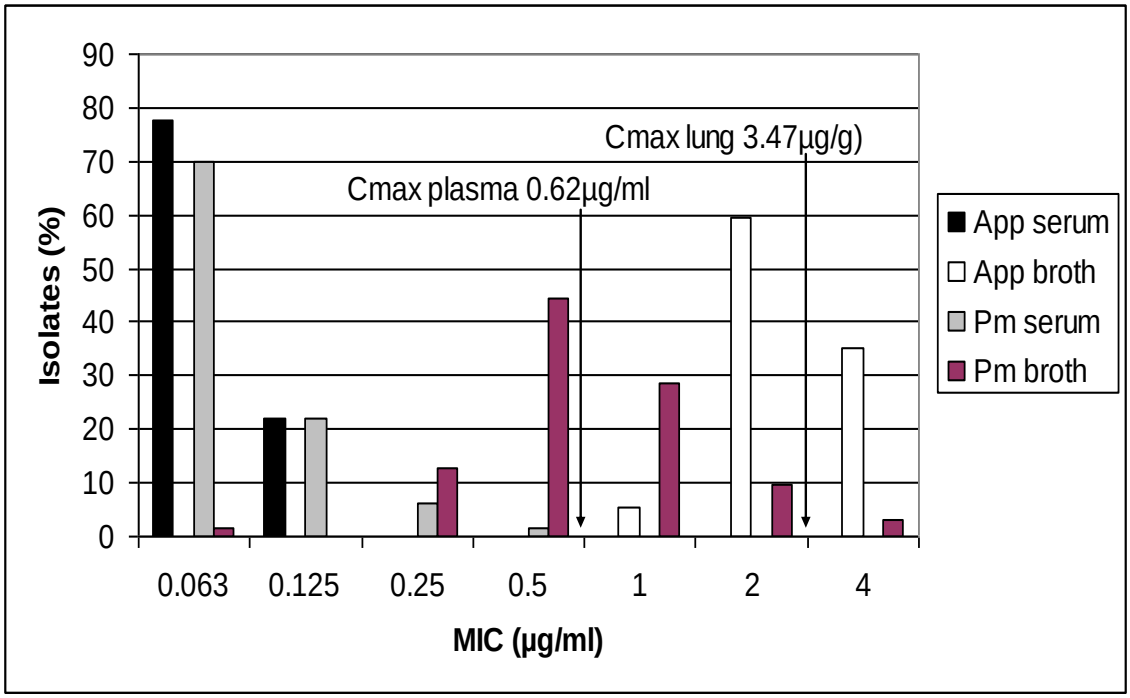
Recent work offers the explanation why the PK/PD integration for certain antimicrobials and certain infections like *A. pleuropneumoniae* and *P. multocida* do not work. *Illambas *et al* (2008) compared the MICs of tulathromycin against *P. multocida* and *Mannheimia haemolytica* from cattle, grown in broth and serum. There was a 50 fold decrease in MIC when the bacteria were grown in serum (see Table 2.26).

Table 2.26. Comparison of tulathromycin MICs when organisms are grown in broth or serum (*Illambas *et al*, 2008)

Matrix	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC ratio
Broth	2.07	3.33	1.61
Serum	0.04	0.06	1.51
Broth/serum ratio	50	53	-

This effect had been described by Godinho *et al* (2005) for *A. pleuropneumoniae* and *P. multocida* but the significance did not appear to be fully recognised by the authors (see Figure 2.8). The method is not prescribed by CLSI. Their method has been devised to overcome growth problems, frequently by the addition of growth enrichment substances for various bacteria and to develop a standard method. They did not necessarily develop a method to assess the pharmacodynamic activity of antimicrobials for PK/PD analysis. All the bacteria, which have MICs derived in serum, fall below the tulathromycin Cmax in plasma, whereas the majority of isolates with MICs grown in broth are above.

Figure 2.8. Comparison of MICs of tulathromycin against *A. pleuropneumoniae* and *P. multocida* in broth and serum (Godinho *et al*, 2005)



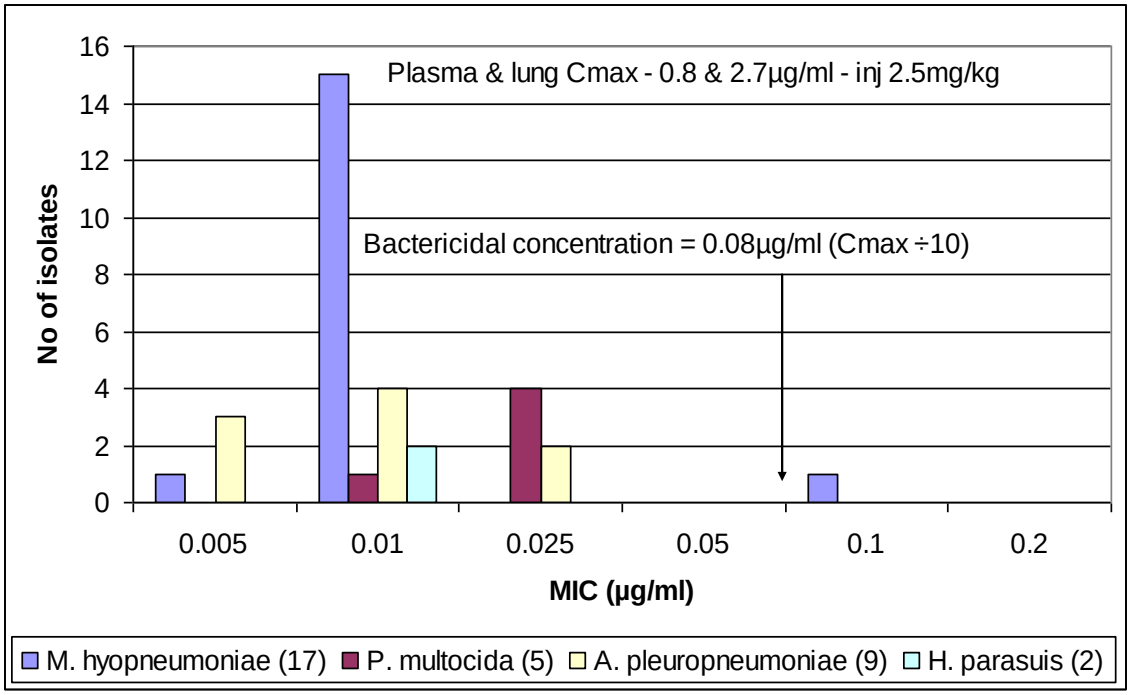
Assessing plasma and lung PK/PD relationships with regard to antimicrobial susceptibility patterns and resistance development

When antimicrobials are used against these respiratory bacteria they may select for resistance. This resistance development may be caused by a variety of processes depending on the type of antibiotic and the bacterium involved but commonly involve gene transfer from plasmids, or selection of mutant strains. Over time, a susceptibility/resistance pattern develops. The so-called 'driver' for this selection of resistance is the antimicrobial concentration achieved in the target tissue or fluid.

Enrofloxacin:

Hannan *et al*, (1989), at around the time of the introduction of the product, found the major porcine respiratory bacteria and *M. hyopneumoniae* MICs for enrofloxacin to be low. The majority of isolates had values of Cmax plasma/MIC greater than 10 at ≤0.08µg/ml, suggesting that the majority of these would be treated effectively (Figure 2.9) and the bacteria would be killed. Intermediate effects, such as bactericidal or bacteriostatic effects, could be expected up to 0.8µg/ml but elimination is unlikely to occur at these higher MIC levels.

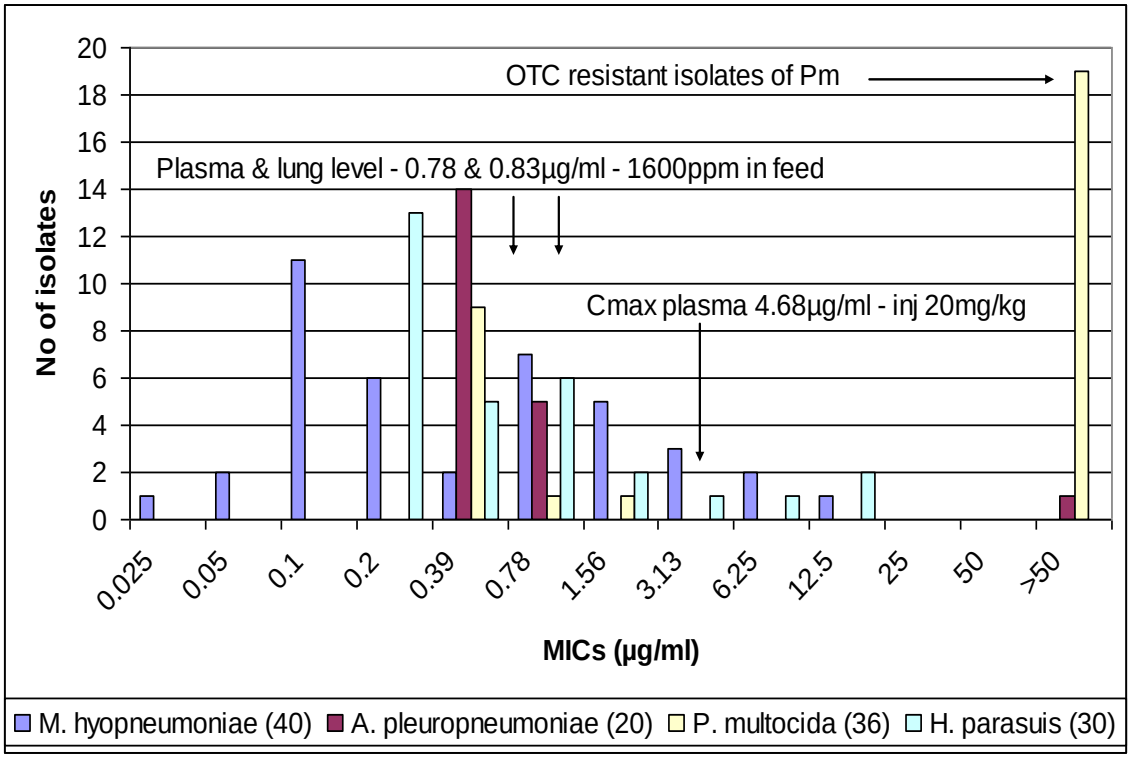
Figure 2.9. Susceptibility pattern for enrofloxacin against common respiratory pathogens (Hannan *et al,* 1989)



Oxytetracycline:

There are two susceptibility peaks for *M. hyopneumoniae*, suggesting some mutation at approximately 0.39µg/ml, so this figure can be considered the epidemiological cut-off value (ECOV) or 'wild type' breakpoint. The next ECOV is at about 1.56µg/ml, which corresponds to the breakpoint for the respiratory bacteria, especially *P. multocida*. The ECOV is considered the normal MIC pattern of isolates prior to exposure to an antibiotic. There is then a major MIC shift to >50µg/ml where there is true resistance (Figure 2.10).

Figure 2.10. Susceptibility patterns for oxytetracycline against common respiratory pathogens (Inamoto *et al*, 1994 (*M. hyopneumoniae*); Pijpers *et al*, 1989 (*A. pleuropneumoniae, P. multocida*); Martin-de la Fuente *et al*, 2007 (*H. parasuis*))

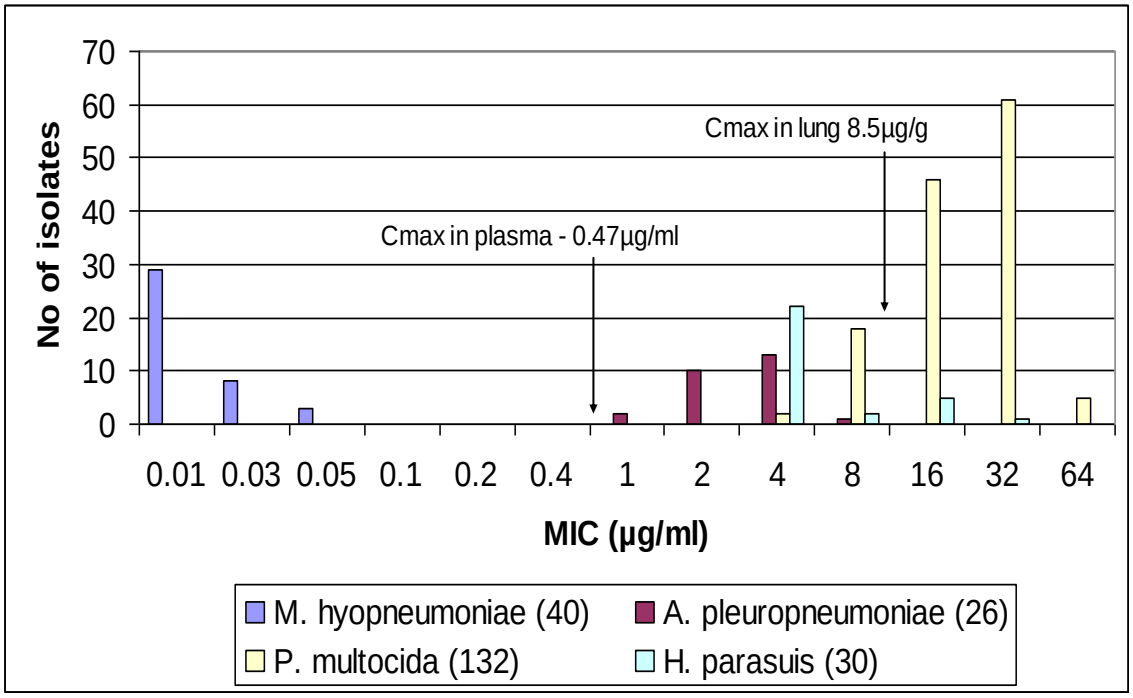


Plasma concentrations seem to be the most important PK factor also for oxytetracycline.

Tiamulin:

The susceptibility pattern of the respiratory pathogens against tiamulin is markedly different in comparison with those against enrofloxacin and oxytetracycline with *M. hyopneumoniae* having a wild type pattern up to 0.05µg/ml and the next is approximately 8.0µg/ml for *A. pleuropneumoniae* and *H. parasuis*. This fits well with the clinical trial results with tiamulin administered in the drinking water at 180ppm and the resulting plasma and lung concentrations. *Pasteurella multocida* seems to be not so susceptible at MICs greater than 8.0µg/ml (Figure 2.11). Tiamulin is less active in acid environments like tulathromycin and it is was thought highly likely that there may be a tulathromycin-like effect if MICs were determined in serum. However, this was shown not to be the case (Pridmore *et al*, 2011) when *A. pleuropneumoniae* was grown in 100% serum.

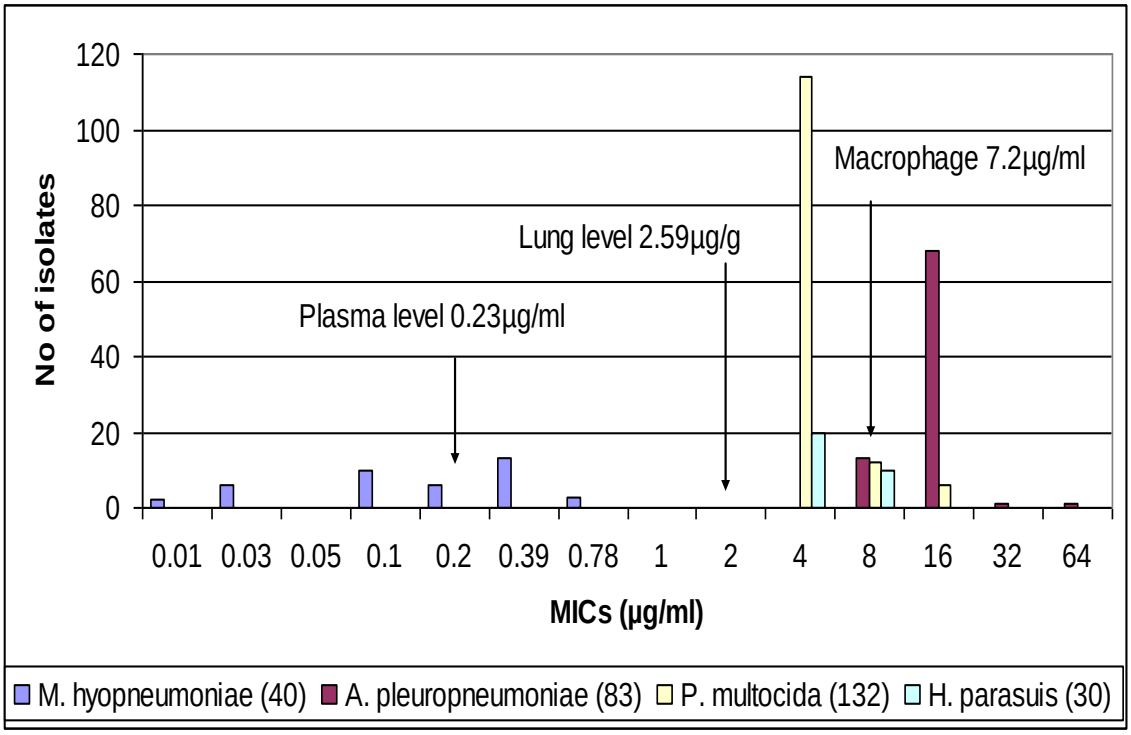
Figure 2.11. Susceptibility pattern for tiamulin against common respiratory pathogens (Inamoto *et al*, 1994 (*M. hyopneumoniae*); Vera-Lizarazo *et al*, 2006 (*P. multocida*); *Casals *et al*, 1990 (*A. pleuropneumoniae*); Martin-de la Fuente *et al*, 2007 (*H. parasuis*))



Tilmicosin:

Tilmicosin has a divided MIC pattern similar to tiamulin, e.g. one for *M. hyopneumoniae*, which dips at 0.78µg/ml and is similar to plasma concentrations, and a second one for respiratory bacteria, which has an ECOV and clinical breakpoint at approximately 8.0µg/ml, which is similar to the macrophage concentration. The lung concentration is low in comparison with the bacterial MICs, hence the suggestion that Tilmicosin concentration in the macrophage might be significant. Honeybourne and Baldwin (1992) reviewed the site concentration effects of antimicrobial agents in the lung and pointed out that macrolides do concentrate in alveolar macrophages with approximately 50% of cell associated macrolide being in the lysosomes. It also has a very low pH and is likely to cause a high level of ionisation of the macrolide. This is therefore likely to reduce the antibacterial activity of the macrolide, rather than enhance it (Lees *et al*, 2006; *Toutain, 2008). It is highly likely that tilmicosin acts in the same way as tulathromycin and a completely different PD picture would be obtained if the MIC determinations were carried out in serum. Artificial challenge studies with known MIC bacteria have been carried out but the majority of isolates had MICs of ≥16µg/ml (Shryock – personal communication) hence the CLSI breakpoint recommendations.

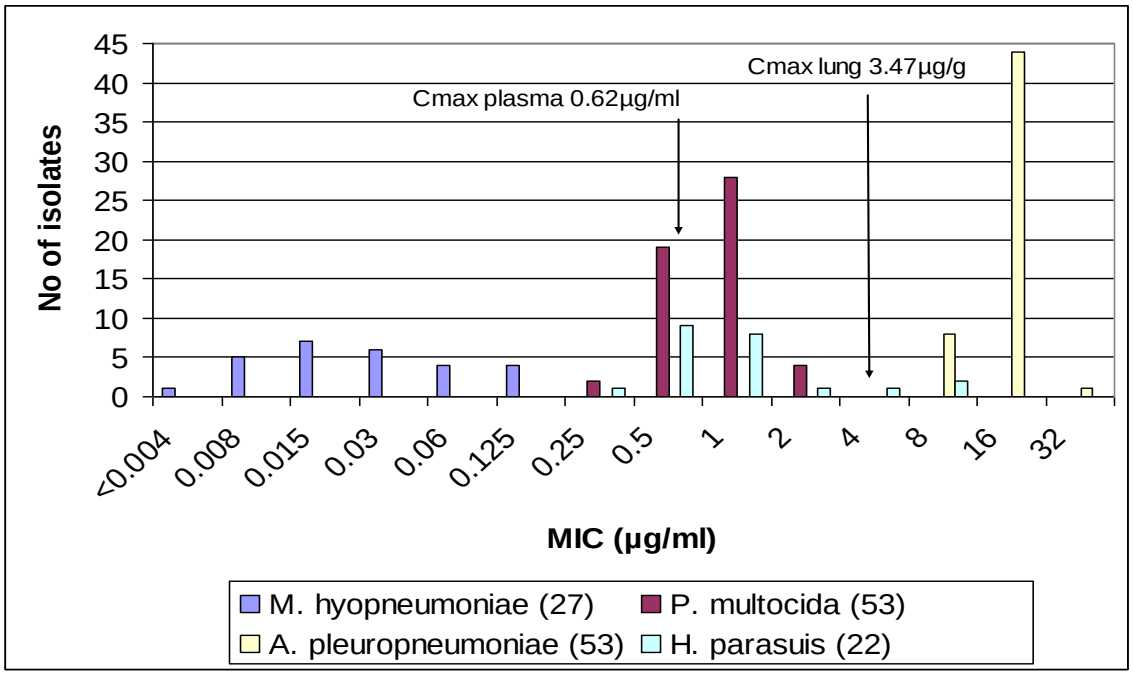
Figure 2.12. Susceptibility pattern for tilmicosin against common respiratory pathogens (Inamoto *et al*, 1994 (*M. hyopneumoniae*); Matter *et al*, 2007 (*A. pleuropneumoniae*); Vera-Lizarazo *et al*, 2006 (*P. multocida*); Martin-de la Fuente *et al*, 2007(*H. parasuis*))



Tulathromycin:

Tulathromycin has three separate peaks, one associated with *M. hyopneumoniae* with an ECOV at 0.25µg/ml, one associated with *P. multocida* and *H. parasuis* at 2.0µg/ml and the third at 32µg/ml associated with *A. pleuropneumoniae*. The first two peaks correlate well with plasma and lung levels. The third peak is much higher than even lung concentrations, yet clinical efficacy has been observed. This may be due to the variations caused in determining the relevant MICs for *A. pleuropneumoniae,* due to the different culturing methods employed (Godinho *et al*, 2005; *Illambas *et al*, 2008). Macrophage concentrations are thought to be unlikely to play a role as they are similar to lung concentrations, unlike tilmicosin. There are not enough MIC data to demonstrate resistance patterns to tulathromycin except for *H. parasuis*. Tulathromycin MICs derived using serum could put most of the bacteria into the plasma susceptible range (Figure 2.8).

Figure 2.13. Susceptibility pattern for tulathromycin against common respiratory pathogens (Godhino *et al*, 2005; Godhino, 2008)



Conclusions

Our understanding of PK/PD relationships in comparison with clinical effect are still developing as more information becomes available. Treatment outcome for *M. hyopneumoniae* appears to correlate well with plasma concentrations. Where there is little difference between plasma and lung concentrations it is less important for respiratory infections and plasma PK/PD relationships are relevant (*Toutain, 2008) but for those compounds that concentrate in lung tissue, as determined by homogenisation of the lung tissue, there seemed to be a potentially significant relationship between lung concentrations and efficacy. The role of antibiotic concentrations in neutrophils and alveolar macrophages are conceivably more significant against bacterial infections, especially for tilmicosin (see Table 2.27 below) where the macrophage concentrations markedly exceed lung concentrations. Neutrophils are also highly mobile and can attack invading bacteria and engulf them. The presence of the antimicrobial drug may therefore be of great significance. Tiamulin's estimated leucocyte concentration is almost identical to lung concentration and tulathromycin's are also similar. However, these concepts have been challenged by *Toutain (2008), particularly on the basis that the lysosomes are acidic; the drugs are ionised and less likely to be effective. This generalisation has been refuted by *Stuart *et al* (2008) where they have shown it does vary for different macrolides and that endosomal pH in alveolar macrophages stays neutral for tilmicosin and tylvalosin but not for tylosin. This is still an area of contention, but some of the original work using 100% serum as a culture medium (*Illambas *et al*, 2008) may be less conclusive regarding the medium but more as a result of increased pHs found in serum (Lees – personal communication).

Care must be taken to include all bioactive metabolites in considering efficacy. Therefore for some drugs it is better to use a microbiological assay rather than HPLC, especially when the drug is extensively metabolised, such as tiamulin, which produces a number of active metabolites (Lykkeberg *et al*, 2007).

Table 2.27. Comparison of leucocyte and lung concentration and epidemiological cut-off value for various antimicrobial drugs (see Table 2.11 for PK data) (Analysis DB)

Antimicrobial	Plasma Conc (µg/ml)	Estimated leucocyte concentration (µg/g)	Leucocyte /plasma concentration ratio	Lung conc (µg/g)	Epidemiological cut-off value (µg/ml)
Tilmicosin 400ppm feed	0.039	**7.2**	184	1.69	**8.0**
Tiamulin 180ppm water	0.47	**8.6**	18.2	8.5	**8.0**
Tulathromycin 2.5mg/kg inj	Cmax 0.62 Mean 6days 0.08	PMNs **1.3-10.3** AMs **0.6-5.0**	PMNs 16.6 AMs 8.1	Cmax 3.47 Mean 6days 2.4 Mean 15days 1.7	**4.0**

Key: - PMNs = polymorphonucleocytes; AMs = alveolar macrophages

The pharmacodynamics of some fastidious bacteria, such as *A. pleuropneumoniae*, is poorly understood, as their MICs vary according to the culture method used. The CLSI method appears to give very high MICs for some antibiotics but their method is not trying to relate these to MICs for PK/PD determination but to ensure a consistent reproducible standard. Therefore efficacy can only be established by artificial challenge studies with known MIC bacteria and these are part of the CLSI standards for interpretation of MICs. Susceptibility/resistance patterns and ECOV determinations require that the antimicrobial drugs have to be available for some time to allow for resistance development to occur in the field and suitably large numbers of isolates need to be tested, but this is becoming a very useful approach to correlate PK with PD.

In conclusion, for those antimicrobials that **accumulate in lung tissue**, lung pharmacokinetics may still have an important role as a **surrogate marker** in establishing PK/PD relationships and clinical efficacy against bacterial respiratory infections. The role of **alveolar macrophage** concentrations also appears to have an important effect, when the antimicrobial concentrates in them to a very high degree, such as tilmicosin and tiamulin, but it is less easy to measure. Doubts over the different methods of **determining MICs using serum** are growing (Pridmore *et al*, 2011). Therefore, it is necessary for further work and clarification, before this is proven a likely route of success for PK/PD integration improvement with free drug in plasma.

Chapter 3. PK/PD integration for enteric diseases

3.1 Introduction

The PK parameters and variables of antimicrobial drugs used for the treatment of intestinal infections of the pig, and there relationship with the PD of enteric bacteria have been described (*Burch, 2005a; *Burch, 2005b; Burch, 2006). There are three major regions of clinical significance; the upper and mid small intestine (duodenum and jejunum) for *E. coli* infections, the lower small intestine (ileum) for *L. intracellularis* infections and the large intestine (colon) for *B. hyodysenteriae* and *B. pilosicoli* infections. There are very limited data on gut concentrations achieved by antibiotics. There is, however, some information on faecal or colonic contents concentration, as these data are now required for regulatory purposes, regarding environmental risk assessments, establishing microbiological maximum residue limits and more recently for PK/PD integration regarding efficacy. Due to the shortfall in PK data regarding the higher parts of the intestine particularly for ileitis claims and for *E. coli* infection claims, it was thought to be useful to develop a model to help correlate likely PK concentrations in the gut with the copious amount of PD data available regarding gut infections. This section reviews the PK data of gut antimicrobial drugs in the pig and establishes a model for PK estimations in the absence of factual data.

3.2 Creating a PK model of the gastro-intestinal tract (DB)

Clemens *et al* (1975) investigated the passage of food along the intestinal tract of adult pigs, using non-absorbed liquid markers (polyethylene glycol and **chromium-labelled ethylenediaminetetraacetic acid (Cr-EDTA).** These were singly dosed by stomach tube mid-meal to adult pigs fed every 12 h. The transit rate and percentage of dose in each section of the gut (stomach, upper small intestine, mid small intestine, lower small intestine, large intestine) were determined, following sequential slaughter of the pigs at 0, 2, 4, 8, 12, 16, 20, 24, 38, 50 and 60 h post-feeding.

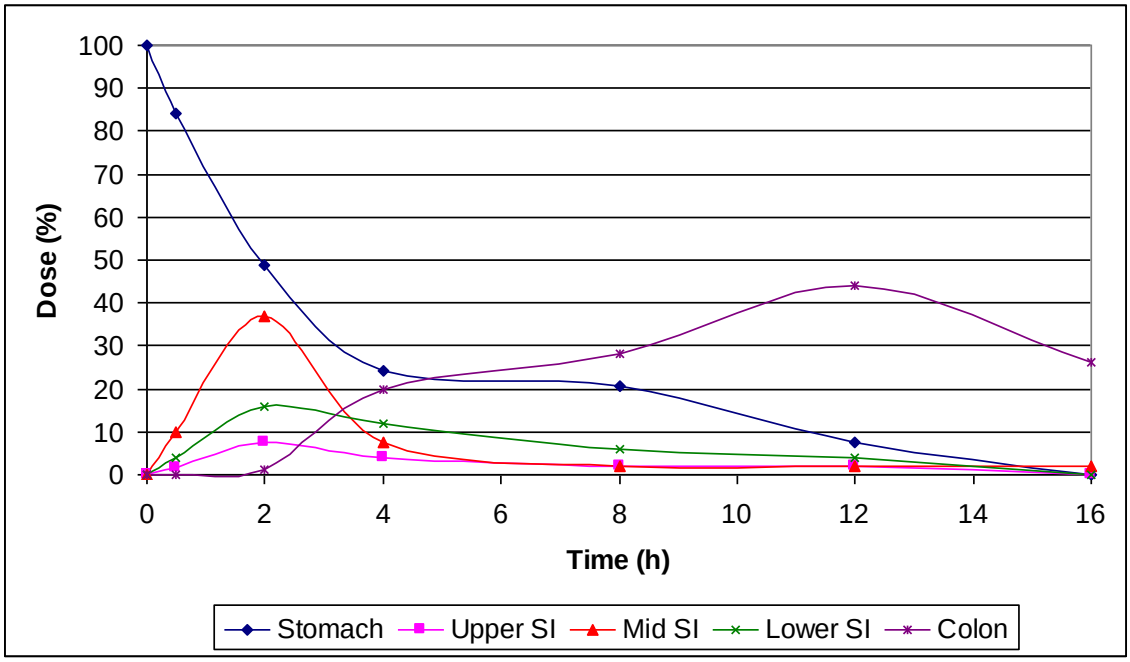
Based on the data of Clemens *et al* (1975) the pharmacokinetic variables, Cmax, AUC and rolling mean (RM) could be calculated for each section of the small intestine over a 12 h time period after dosing (*Burch, 2005b). The AUC was calculated using the trapezoidal method and the RM was calculated by dividing the AUC by 12h. As the product did not enter the mid colon for two hours, a 2-14 h time period was used in the calculations to compensate. To estimate the AUC 24 h, the 12h AUC was multiplied twice or alternatively the RM over 12 h was multiplied by 24 or, which reflects the pig's normal feeding of at least twice daily.

The intestinal transit data based on Cr-EDTA measurement and PK results are shown in Table 3.1 and Figure 3.1.

Table 3.1. Cmax, AUC 12 h and RM and gut concentration ratio for Cr-EDTA in gut contents of the pig related to concentration in the mid-colon (Clemens *et al,* 1975; *Burch, 2005b) (Calculations DB)

	Cmax (% dose)	AUC 12 h (% dose h)	Rolling Mean (% dose)	Ratio (% dose)
Upper small intestine (duodenum)	7.5	38.8	3.2	11.4
Mid small intestine (jejunum)	37	108	9.0	31.8
Lower small intestine (ileum)	16	100	8.3	29.4
Large intestine 2-14 hours (mid-colon)	44	340	28.3	100

Figure 3.1. Dose (%) found in the stomach, upper, mid and lower small intestine and colon contents against time over 16h (Clemens *et al,* 1975; *Burch, 2005b)

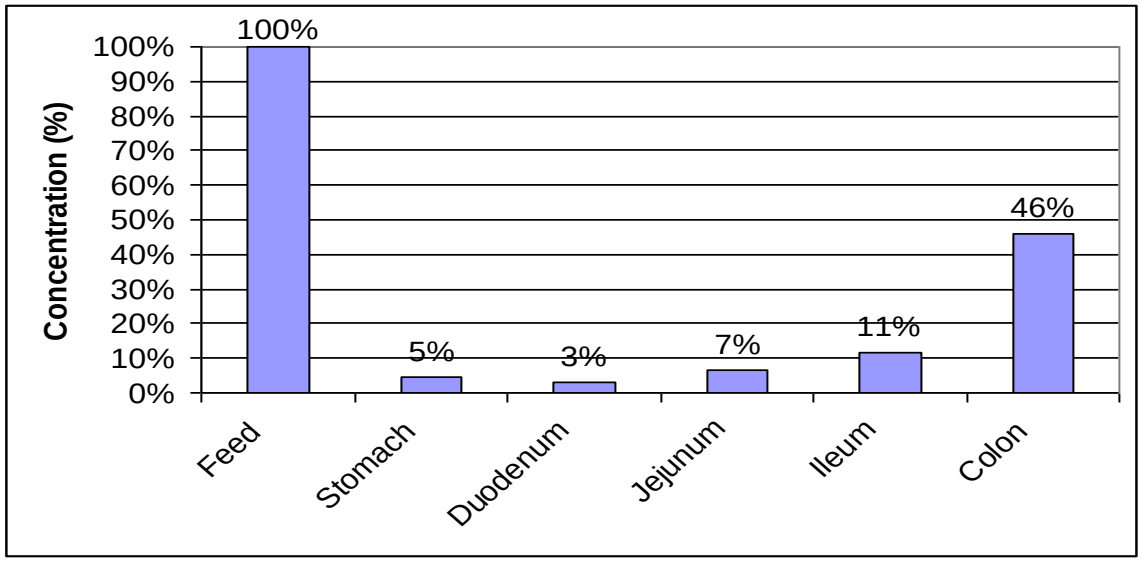


The first samples were taken at 2 h and only relatively low drug concentrations were present in the upper small intestine, with a Cmax of 7.5 % dose being estimated, although 51% of the dose had passed out of the stomach by this time and 76% by 4 h. The dose recovery from the upper small intestine, in the 12 h period, was only 38.8%, indicating that a substantial amount of the dose was not accounted for. The jejunum Cmax was much higher at 37 % and AUC 12h was 108% of the dose, suggesting a partial over estimation. The ileum had a Cmax of 16 % dose and 100% of the dose was identified. The Cr-EDTA accumulated in the large intestine (mid colon) and concentrated there giving a Cmax of 44% of dose and AUC 12 h of 340 % of dose or at concentrations approximately 3.4 times the ileum RM concentration.

Refining the gastro-intestinal tract drug concentration model (Refinement DB)

There are a number of parts of the Clemens *et al* (1975) work and *Burch (2005b) model, which would appear to underestimate drug concentrations in the duodenum. The rolling mean and AUC values for the ileum were 29.4% of the colon values over a 12 hour period and appear to be similar to the results (24%) from *DeGeeter *et al* (1980) who fed lincomycin at 220ppm in the feed to 30kg pigs continuously for 23 days (see Figure 3.2). The sampling time was not reported. The concentration in the stomach was low, suggesting that much of the lincomycin had already passed on from the stomach, possibly four hours or more after feeding, and levels in the duodenum and jejunum were also low, but were higher in the ileum.

Figure 3.2. Lincomycin concentrations (% of feed concentration) in the gastrointestinal tract following feeding at 220ppm for 23 days (*DeGeeter *et al,* 1980)

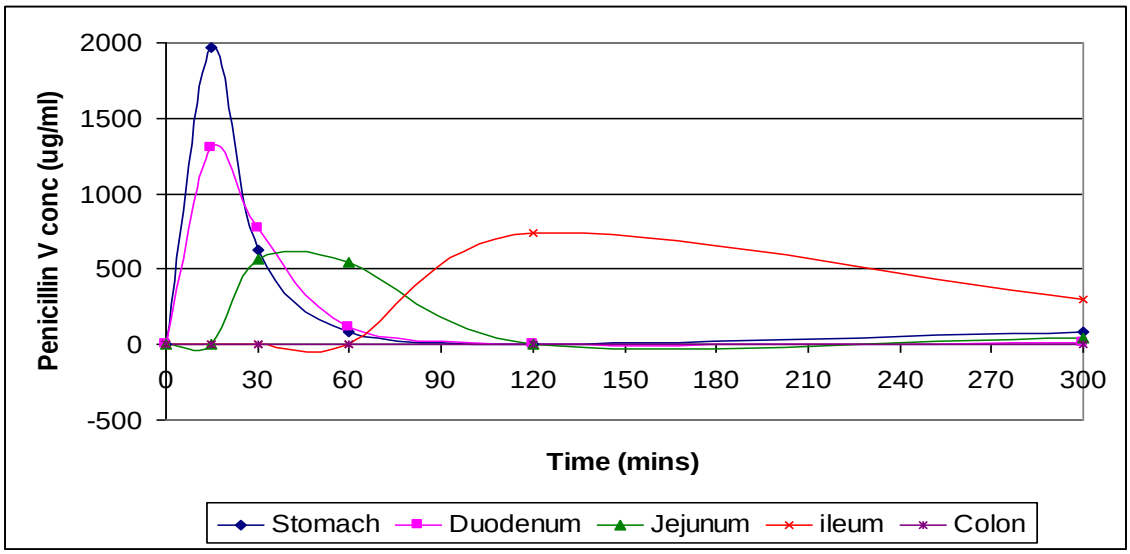


Gastric emptying time in the pig has been reported by a number of authors. It can be slowed by age (Snoeck *et al,* 2004), feeding (Casteel *et al,* 1998) and by formulation (Davis *et al,* 2001). For example liquids passed through more quickly than micro-pellets. Suckling pigs had a faster gastric emptying time than weaned pigs, two days and two weeks after weaning, but by three weeks after weaning, pigs had a similar gastric emptying time to adults (Snoeck *et al*, 2004) as demonstrated in the Clemens *et al* (1975) work. Transit-time data for the small intestine was limited, usually by the complexity of the pig's intestines on radiography and also by inadequate and prolonged intervals between measurements. Davis *et al* (2001) reported a transit time of 3-4 hours for liquids and Snoeck *et al* (2004) reported >7 hours (90% clearance) for micro-pellets in meal.

McKellar *et al* (1987) reported on gastric emptying and passage along the gastrointestinal tract of procaine penicillin given as an aqueous suspension orally at a dose of 15.9mg/kg to 60-80kg pigs, which had been previously fasted for 12 hours.

Samples were taken from stomach, duodenum, jejunum, ileum and colon at 15, 30, 60, 120 and 300 minute intervals from one pig per time point.

Figure 3.3. Concentrations of penicillin (µg/ml) found in the gastrointestinal tract of pigs following administration of a single oral dose of procaine penicillin suspension at 15.9mg/kg (McKellar *et al,* 1987)

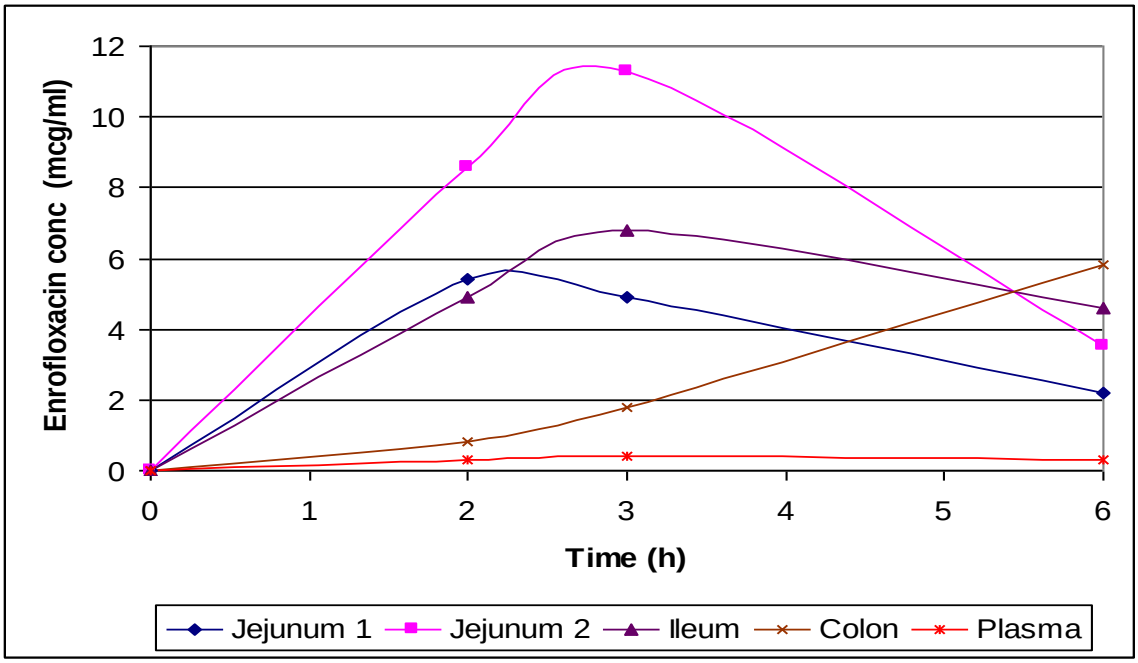


Substantial quantities of the penicillin in suspension entered the duodenum within 15 minutes of administration and into the jejunum by 30 minutes and ileum by 2 h. A comparatively steady concentration was achieved in the ileum. Concentrations in the colon were very low (<0.7µg/ml), due to the absorption and breakdown of the penicillin in the gastro-intestinal tract. These data confirm that drug concentrations in the duodenum should be higher within the first 2h post dosing. Wiuff *et al* (2002) studied the pharmacokinetics of enrofloxacin in unfasted grower pigs, 8-10 weeks of age, following administration by injection and orally by intubation at a dosage of 2.5mg/kg, and administered daily for 5 consecutive days. Plasma, gut and gut content samples were taken at 2, 3, and 6 h after the final treatment (Table 3.2 and Figure 3.4) from four pigs for each time point at necropsy.

Table 3.2. Enrofloxacin concentrations (µg/ml or µg/g) in plasma and gut contents after oral administration at 2.5mg/kg bodyweight (Cmax values in bold) (Wiuff *et al,* 2002)

	2 h	3 h	6 h
Plasma	0.31	**0.40**	0.30
Jejunum 1	**5.4**	4.9	2.2
Jejunum 2	8.6	**11.3**	3.5
Ileum	4.9	**6.8**	4.6
Caecum	1.0	4.2	**4.8**
Colon	0.8	1.8	**5.8**
Rectum/faeces	0.6	0.3	**2.3**

Figure 3.4 Enrofloxacin concentrations (µg/ml or g) in plasma and gut contents after oral administration at 2.5mg/kg bodyweight (Wiuff *et al,* 2002)



Concentrations of enrofloxacin in plasma and intestinal contents were similar for intra-muscular and oral administrations, suggesting that after intra-muscular dosing, enrofloxacin is excreted via the bile or other secretions relatively unchanged. The metabolite ciprofloxacin was present only at low levels. The upper small intestine (jejunum 1) peaked at 2 h but jejunum 2's (mid small intestine) Cmax was at 3 h, and reached twice the jejunum 1 concentration. The ileum concentration peak also occurred at 3 h and remained relatively high at 6 h. Colon, caecum and rectum concentrations were maximal at the final sampling time (6 h). These data were similar to the findings of Clemens *et al* (1975), except the mid-jejunal peaks were slightly later at 3 h.

By measuring the concentration of the intestines 2 h after dosing, it was assumed that a substantial amount of the dose had already passed the duodenum and jejunum and into the ileum. An estimate was made by the author (DB), using the same AUC fraction in the duodenum that was lost from the stomach in that 2 hour period and a Cmax of 63.5% of the dose was estimated at a time (Tmax) of 30 minutes and then 100% of the dose would have been recovered. This suggests that the time interval for making these gut calculations should be reduced below 2 hours to give more accurate assessments higher up the gut (see Table 3.2 and Figs. 3.3 and 3.4) and possibly at more frequent intervals to increase the recovery percentage to 100% (Table 3.3 and Figure 3.5)

Table 3.3. Revised Cmax, AUC 12 hours and Rolling Mean (RM) and concentration ratio for Cr-EDTA in gut contents of the pig (Revision DB)

	Cmax (%)	AUC 12 h (% h)	Rolling Mean (%)	Ratio (%)
Upper small intestine (duodenum)	7.5	38.8	3.2	10.7
Upper small intestine (revision estimate)	**63.5**	**100**	**8.3**	**27.7**
Mid small intestine (jejunum)	37	108	9.0	31.8
Lower small intestine (ileum)	16	100	8.3	29.4
Large intestine 2-14 hours (mid-colon)	44	340	28.3	100

Figure 3.5. Dose (%) found in the stomach, upper (plus estimated adjustment), mid and lower small intestine and colon contents against time (Revision DB)

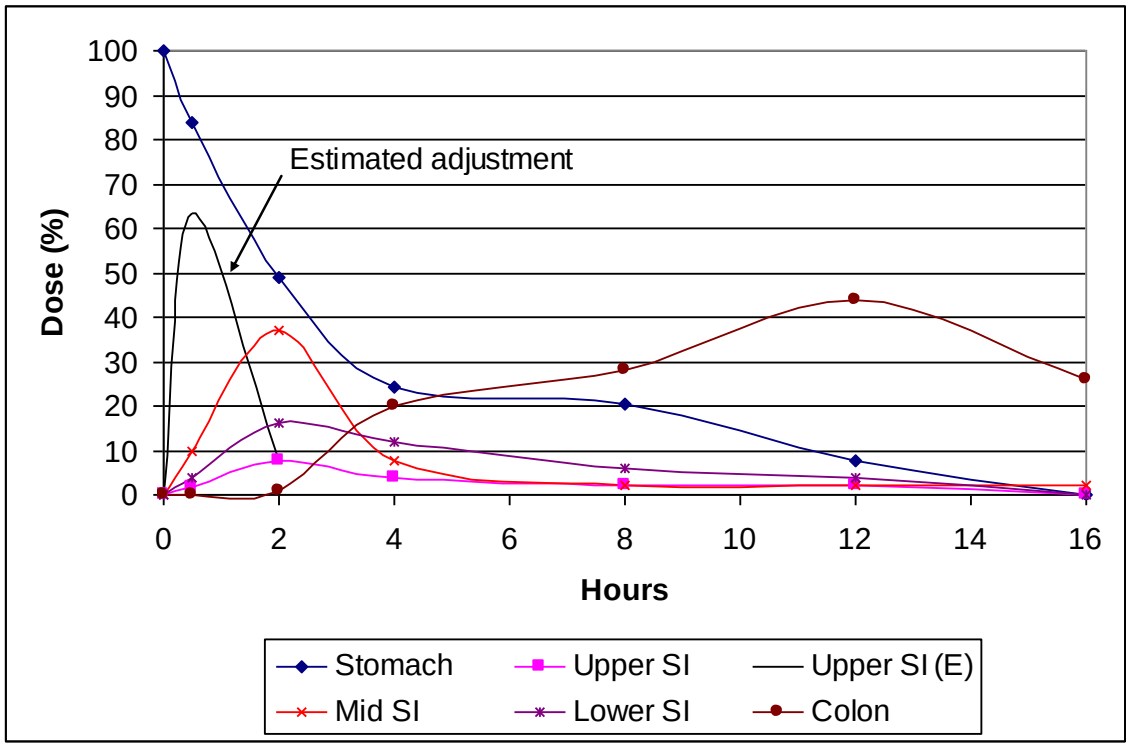
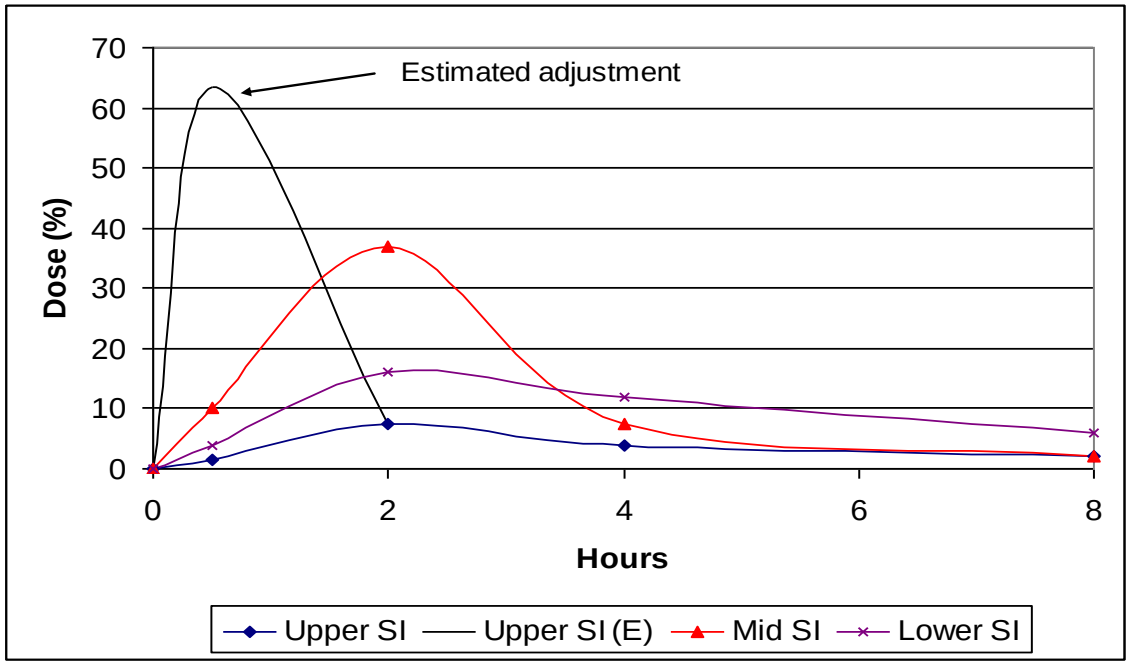


Figure 3.6. Percentage of dose estimated for the upper (plus estimated adjustment), mid and lower small intestine contents against time (Revision DB)



Drug concentrations in the small intestine depend on a number of factors such as gastric emptying, both time and extent, the presence of liquids or food and particle size e.g. granules, the absorption, the excretion of active substance or active/inactive metabolites into the intestine via the bile and other secretions. Breakdown of the product by the acid environment (e.g. penicillin G), enzymes, bacteria and binding also may occur. As food and fluids are absorbed, there is a concentrating effect as the drug transits to the ileum and then into the colon. The environment in the colon could also affect the bioactivity of a drug. It is primarily an anaerobic environment and this limits the activity of some antibiotics, such as the aminoglycosides and some fluoroquinolones. Faecal binding can also have a major impact. For example, enrofloxacin's bioactivity was reduced by 42% according to Wiuff *et al* (2002) comparing microbiological and high pressure liquid chromatography (HPLC) assays. Bacterial breakdown is also likely for some drugs. The dissociation constant (pKa) of some drugs might also be affected in the acid environment of the large intestine with a pH of 6.6 (Hojberg *et al*, 2005).

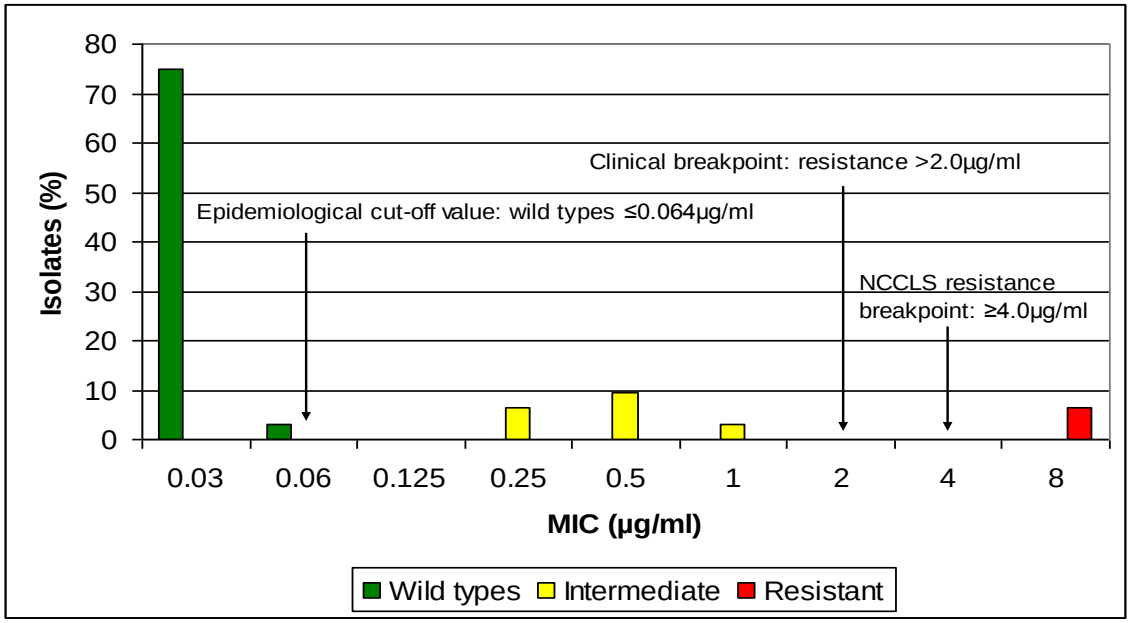
Information on antimicrobial drug concentrations in the faeces or colon contents is often more readily available. These concentrations are less variable than those in the small intestine; hence, they can be used in attempts to determine effective antimicrobial concentrations and the application of PK/PD principles in the small intestine, particularly against infections such as *E. coli*.

3.3 Small intestinal infections – *Escherichia coli*

Kahlmeter *et al* (2003) discussed the harmonisation of MIC breakpoints for antimicrobial susceptibility testing in Europe. Currently, there are two approaches, the

microbiological approach and the **clinical approach**. The former is based primarily on **'wild type' patterns** of MIC distribution; increases in MIC demonstrate the possible development of resistant mutants. **Clinically**, an antimicrobial would be expected to work against the wild type isolates i.e. basic susceptibility. However, depending on the concentration of the antimicrobial drug in the blood or tissue, intermediately susceptible mutants might be still susceptible. These intermediate mutants might be the first step in resistance emergence for some drugs, e.g. the fluoroquinolones, before full resistance development. The setting of these **clinical breakpoints** is complicated and depends on the type of bacteria and the type of antimicrobial drug and also varies between authorities. Hence, there is a need for harmonisation. Terminologically, there are some difficulties, but there is **'epidemiological cut-off value'** for the **wild type** bacterial susceptibility point and **'clinical breakpoint,'** which may be a substantially higher MIC, to reflect the point where clinically successful treatment is likely to occur and where resistance begins. This was graphically demonstrated by Bywater *et al* (2006) (Fig. 3.7).

Figure 3.7. MIC patterns for susceptible wild types (epidemiological cut-off value), intermediate susceptible (clinical breakpoint), and resistant isolates - based on *E. coli* and fluoroquinolone concentrations (Bywater *et al*, 2006)

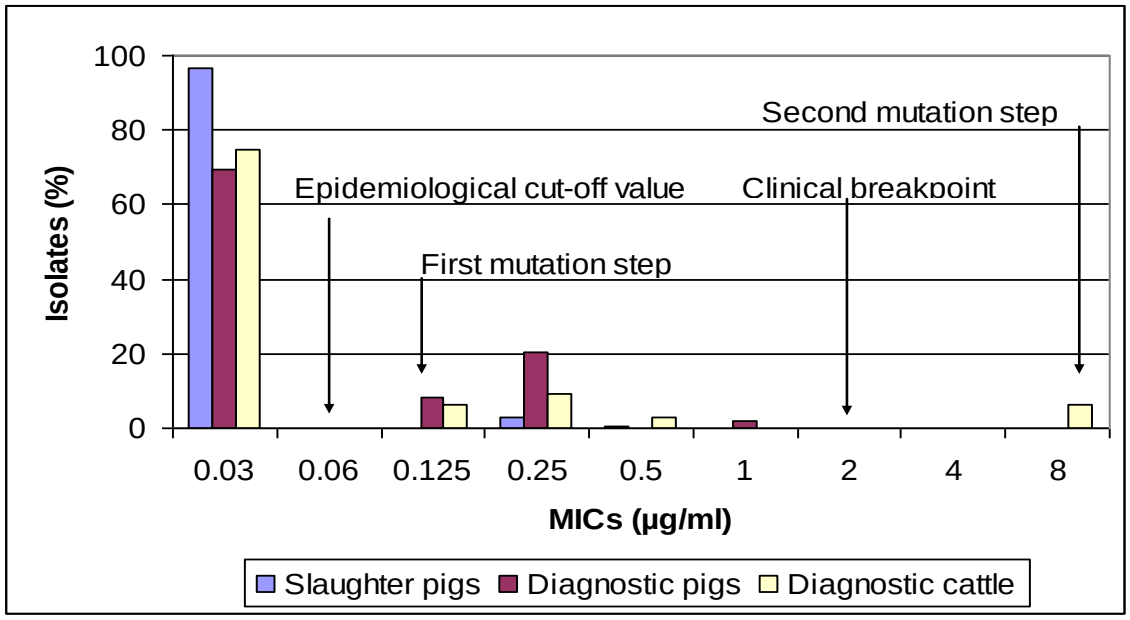


This section reviews MIC patterns of epidemiological slaughterhouse samples, comparing them with clinical isolates submitted for diagnostic purposes and which may have been exposed to antimicrobial drugs prior to submission (Anon., 2005). In the DANMAP 2004 report (Anon., 2005), they used the CLSI antimicrobial resistance breakpoints and these can be considered one dilution higher than the clinical breakpoint. Examples of antimicrobial gut concentrations, where available, will be compared with the MIC susceptibility patterns.

Enrofloxacin (ciprofloxacin)

From the Wiuff *et al* (2002) data, the Cmax plasma was 0.40µg/ml and 11.3µg/ml in the mid small intestine after oral administration of 2.5mg/kg dosage. For fluoroquinolones, to obtain the optimum bactericidal effect, a Cmax 10 times the MIC i.e. concentrations of 0.04 and 1.13µg/ml respectively, is normally proposed or 100-120 times the AUC 24h (Toutain, 2003). These data were not available in the Wiuff *et al* (2002) study, as concentrations were studied only over 6 h. However, the rolling mean figure over 12 h can be used, assuming the pig is dosed twice a day, and multiplied by 24 to give the AUC over 24 hours.

Figure 3.8. Ciprofloxacin MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) and cattle (32) (Anon., 2005)

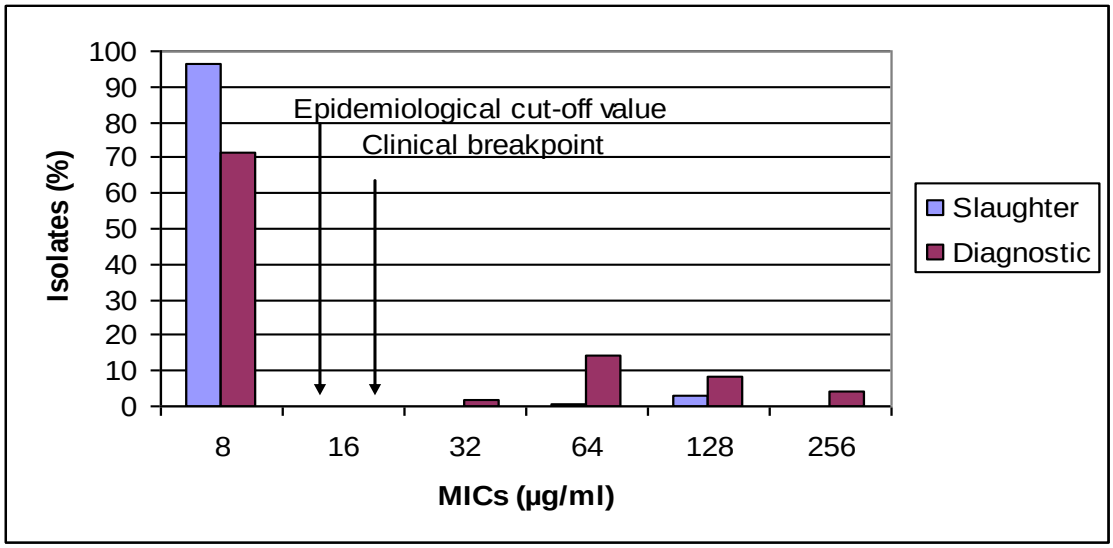


These data from slaughter house pig samples and diagnostic *E. coli* isolates from porcine and bovine diagnostic cases demonstrate the classic fluoroquinolone resistance pattern. The ECOV, where the last of the wild type pattern forms is ≤0.06µg/ml, which is almost attained by $1/10^{th}$ of the plasma Cmax and is reached following injection. This demonstrates that a good bactericidal effect is likely, even in a systemic infection. The next cluster of MICs (0.125-1.0µg/ml) has had a first step mutation and is nalidixic acid (quinolone) resistant. The range of concentrations between 0.06 to 2.0µg/ml is termed the mutant selection window (MSW) (Drlica, 2003). An almost identical pattern is shown by the porcine strains for nalidixic acid and ciprofloxacin except for the value of the MIC (see Figure 3.9). The final cluster of MICs is at 8.0µg/ml and these are considered fully resistant to enrofloxacin.

The clinical breakpoint has been set at >2.0µg/ml and this is exceeded by the gut concentrations but is potentially only 1/6th of the jejunum 2 Cmax gut contents data. At this site, the Cmax would be 11 times higher than the first mutant cluster, potentially giving highly effective bactericidal treatment of these mutants i.e. a mutant prevention concentration (MPC) after oral dosing, if they were situated in the gut only, but not systemically. This has clinical significance in neonatal scours in piglets, as systemic

invasion by *E. coli* can occur. It also highlights the importance of applying the pharmacokinetics to the target site, e.g. gut contents. The bovine diagnostic data was included to demonstrate the next mutation, which has led to full resistance, most likely in calves. However, by using fluoroquinolone concentrations, which kill not only the wild types but also the first step (nalidixic acid) resistant mutants, the likelihood of full resistance development is much reduced (Drlica, 2003).

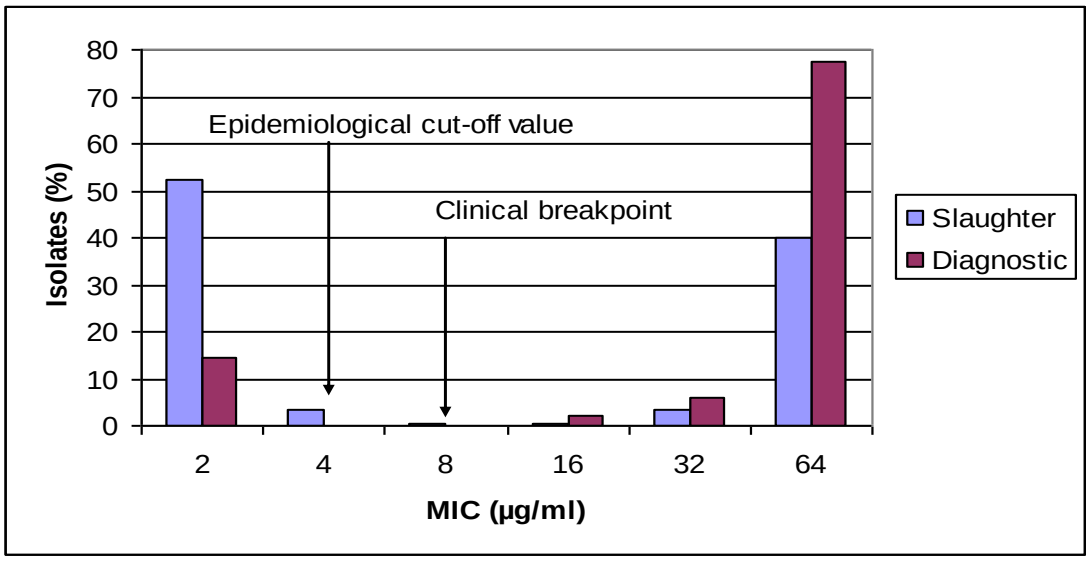
Figure 3.9. Nalidixic acid MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Other absorbable antimicrobial drug MIC patterns

Tetracycline

Figure 3.10. Tetracycline MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Hansen *et al* (2002) recorded the bioactive concentration of chlortetracycline, a primarily bacteriostatic antibiotic, in pig faeces after oral administration in the feed at 800ppm. The bioactive residue was 112µg/g chlortetracycline in the faeces. The usual incorporation rate of chlortetracycline is normally 300-400ppm, which would provide 42-56µg/g in the faeces and approximately 13-17µg/g in small intestinal contents (faecal conc. x 30% based on Clemens *et al*, 1975 and *Burch 2006 model). These figures support the overall susceptibility pattern and also the shift to the right, which can be expected after treatment and an increase in resistance.

Ampicillin and amoxycillin + clavulanic acid

Figure 3.11. Ampicillin MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)

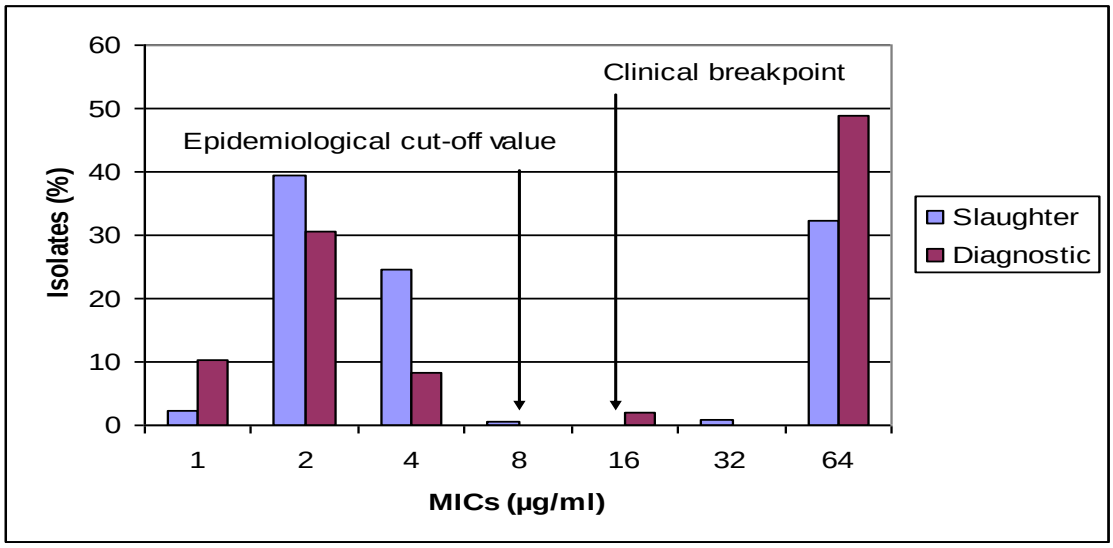
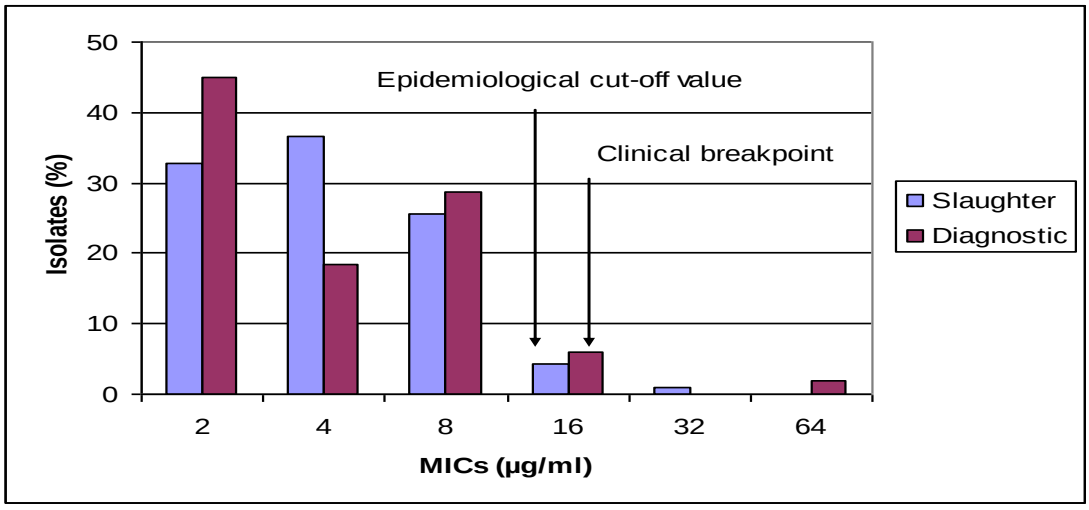


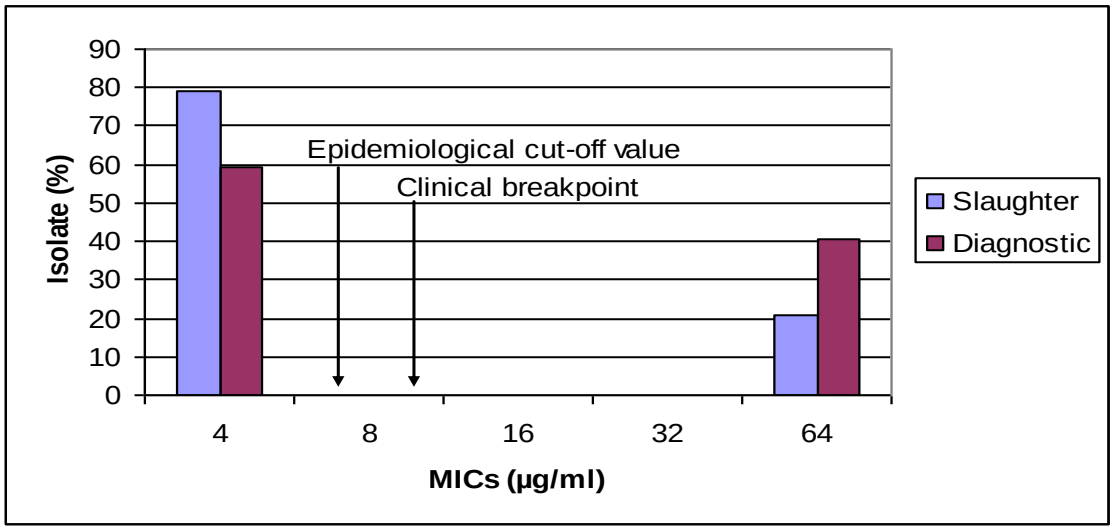
Figure 3.12. Amoxycillin + clavulanic acid (2+1 ratio) MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



The susceptibility profile of ampicillin, a time-dependent bactericidal antibiotic, is distinctive with regard to ECOV and clinical breakpoint. Amoxycillin has a similar activity to ampicillin, but with the addition of clavulanic acid, there is a marked difference in susceptibility profile and resistance reduction. Clavulanic acid is a beta-lactamase enzyme inhibitor, thereby demonstrating the importance of beta-lactamase production by *E. coli* as a resistance mechanism.

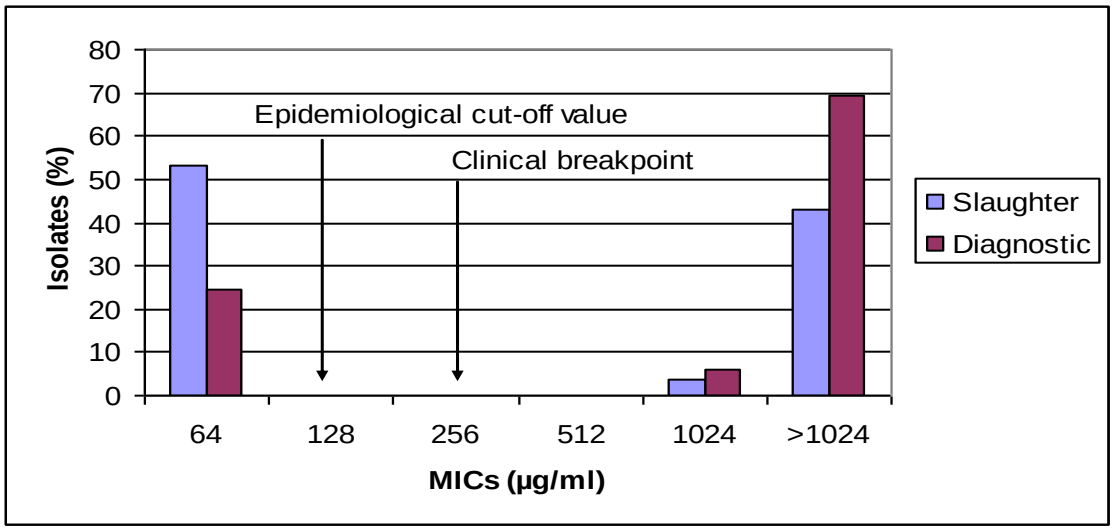
Trimethoprim and sulphonamides

Figure 3.13. Trimethoprim MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Resistance to trimethoprim is usually due to a plasmid or chromosomal production of a binding resistant dihydrofolate reductase enzyme, so that trimethoprim cannot bind to it and interfere with folic acid synthesis by inhibiting a precursor tetrahydrofolic acid (Prescott, 2000b). The resistance, once induced, appears complete. When administered alone, trimethoprim is bacteriostatic; it is only in combination with sulphonamides that it becomes bactericidal.

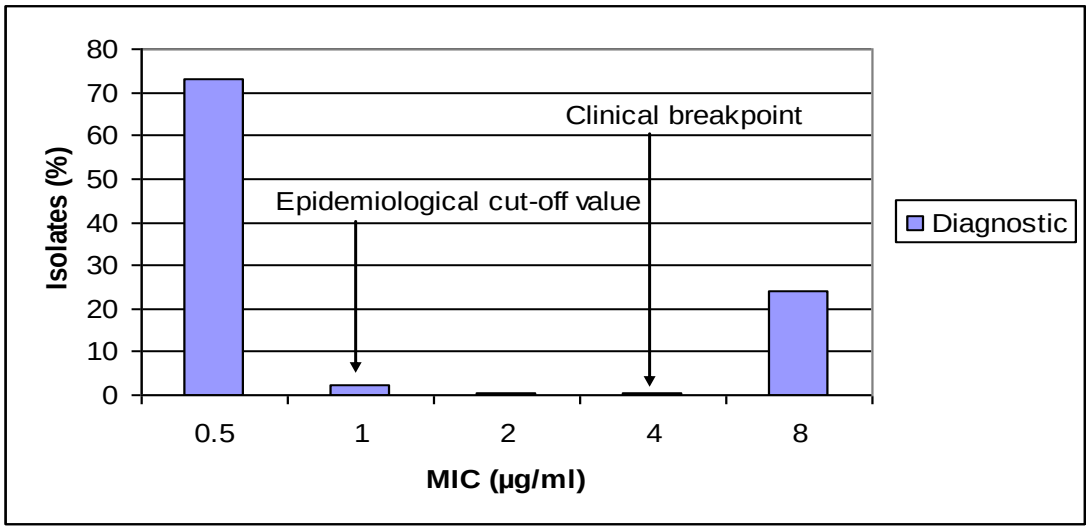
Figure 3.14. Sulphonamide MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Sulphonamides also interfere with folic acid production by competitively blocking para-aminobenzoic acid binding to the enzyme dihydropterate synthetase. Resistance is commonly plasmid mediated and results either from reduced penetration of the bacterial cell or production of sulphonamide-resistant enzymes.

Trimethoprim and sulphonamides are more commonly used together in pig medicine and a MIC pattern is described in Anon. (2006) in porcine diagnostic submissions (Fig. 3.15).

Figure 3.15. Trimethoprim/Sulphamethoxazole (1/20 ratio) MIC patterns against *E. coli* from porcine diagnostic submissions (325) (Anon., 2006)



Due to the synergistic activity between the two compounds, there is a shift to the left in antimicrobial activity, but there is still a relatively clear-cut pattern of susceptibility and resistance.

Pharmacokinetic / pharmacodynamic relationships (Analysis DB)

Unfortunately, there are few pharmacokinetic data to include in PK/PD analyses, except for enrofloxacin and chlortetracycline. Therefore a variety of assumptions have been made, to facilitate a comparative analysis of the absorbable products. The common concentration of the antimicrobial in feed has been used. The bioavailability of the product (amount absorbed) is subtracted and the concentration in the faeces is then multiplied by **1.7 times** the amount of compound after the bioavailability factor has been calculated. This figure is derived from O'Callaghan *et al* (1971) who measured feed intake against faecal output of pigs and showed the correlation was **0.583-0.596: 1 faeces: feed** for pigs weight range 20-90kg bodyweight. The jejunal concentration figure used was **30%** of the faecal concentration (Clemens *et al*, 1975), which provides the rolling mean, which is then multiplied by 24h to give the AUC 24h. This figure is divided by the ECOV or the clinical breakpoint (CBP) to give a figure, to assess the effect of the antimicrobial.

Table 3.4. Estimated antimicrobial pharmacokinetic relationship analysis for absorbed, partially absorbed drugs relative to epidemiological cut-off values (ECOV) and clinical breakpoints (CBP) (Analysis and calculations DB)

Antimicrobial	Feed level* (ppm)	Bioavailability (%)	Faeces conc. (µg/g)	Jejunal conc. (µg/ml)	AUC 24 h (µg.h/ml)	ECOV (µg/ml)	AUC/ECOV (h)	CBP (µg/ml)	AUC/CBP (h)
Enrofloxacin	100	90	-	3.8	92**	0.06	1533	2	46
Chlortetracycline	400	20	56***	14	336	4	84	8	42
Ampicillin	300	30	357	107	2568	8	321	16	161
Amoxicillin/ Clavulanic acid (2/1)	300/ 150	30	357	107	2568	16	161	16	161
Trimethoprim	50	90	8.5	2.6	61	8	8	8	8
Sulphonamide	250	90	43	13	306	64	5	256	1
Trimethoprim/ Sulphonamide (1/20)	300	90	51	15	367	1	367	4	92

*Based on 20ppm = dose of 1mg/kg liveweight per day
** Wiuff *et al*, 2002, estimated data
*** Hansen *et al*, 2002, data

The jejunal AUC 24hours was estimated from the Wiuff *et al* (2002) data. The calculated AUC/ECOV markedly exceeds the 100-120h ratio for bactericidal antimicrobials but the AUC/CBP is only 46h, which demonstrates that it is below the optimal killing effect for fluoroquinolones against *E. coli* in the small intestine.

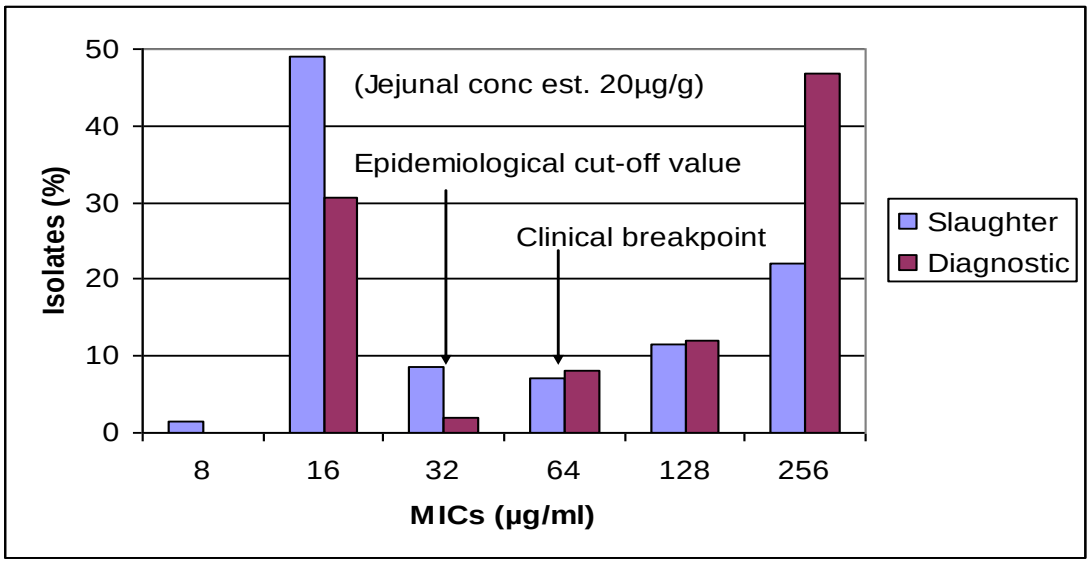
For bacteriostatic drugs, such as chlortetracycline, an AUC/MIC figure should be at least 24 to ensure that the average drug concentration equals the MIC for the 24h period. The AUC/CBP figure and the AUC/ECOV figure exceed 24h (42h), suggesting a good inhibitory effect. Ampicillin is bactericidal and the estimated gut concentrations should be sufficient to kill *E. coli* at the nominated CBC. This would be similar for amoxycillin and clavulanic acid but more strains would be inhibited because of the addition of the beta-lactamase inhibitor. Trimethoprim is bacteriostatic and does not reach the inhibitory AUC/ECOV figure of 24h, nor at the clinical breakpoint. The AUC/ECOV and AUC/CBP figures are even lower for sulphonamides, but when the two drugs are combined there is a very high AUC/ECOV figure of 367h and AUC/CBP of 92h, which would be bactericidal.

Non-absorbed compounds:

Antimicrobial drugs, which are not absorbed from the gastro-intestinal tract, can be expected to follow a similar pattern and concentration relationship to the marker substance (Cr-EDTA) in the Clemens *et al* (1975) study.

Spectinomycin

Figure 3.16. Spectinomycin MIC patterns against *E. coli* from porcine slaughter data (n=208) and porcine diagnostic submissions (49) (Anon., 2005)



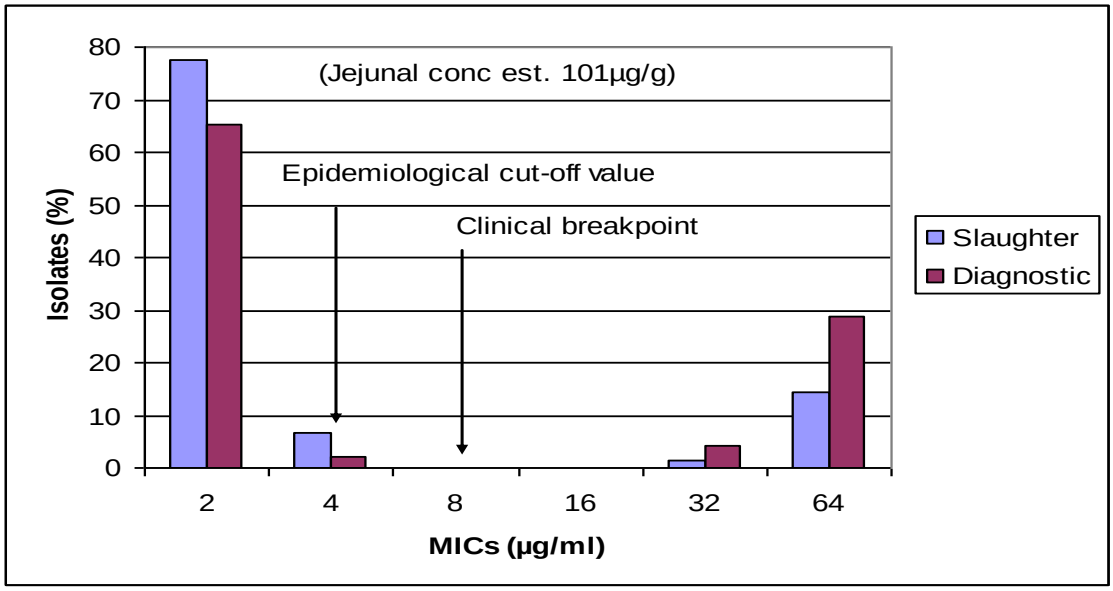
Spectinomycin, an aminocyclitol antibiotic, is commonly used in pigs in combination with lincomycin at 44ppm each. It is primarily bacteriostatic, but at four times MIC may

become bactericidal (Prescott, 2000a). It is predicted that the concentration in faeces should be 67ppm and the rolling mean concentration in the small intestine would be about 20µg/g. This is quite close to the ECOV at MIC of 32µg/ml. The clinical breakpoint (CB) concentration at 64µg/ml looks unattainable by comparison in the small intestine but achievable in the colon. However, the spectinomycin dosage in the soluble formulation is higher than when given, in feed equivalent to 134ppm and could reach closer to 60µg/g in the small intestine and might prove bactericidal for the majority of wild type strains. Resistance is usually chromosomal but there is no cross resistance to the aminoglycosides. It is mainly gut active but some drug, 10-20% (Anon., 2000) is absorbed.

Neomycin

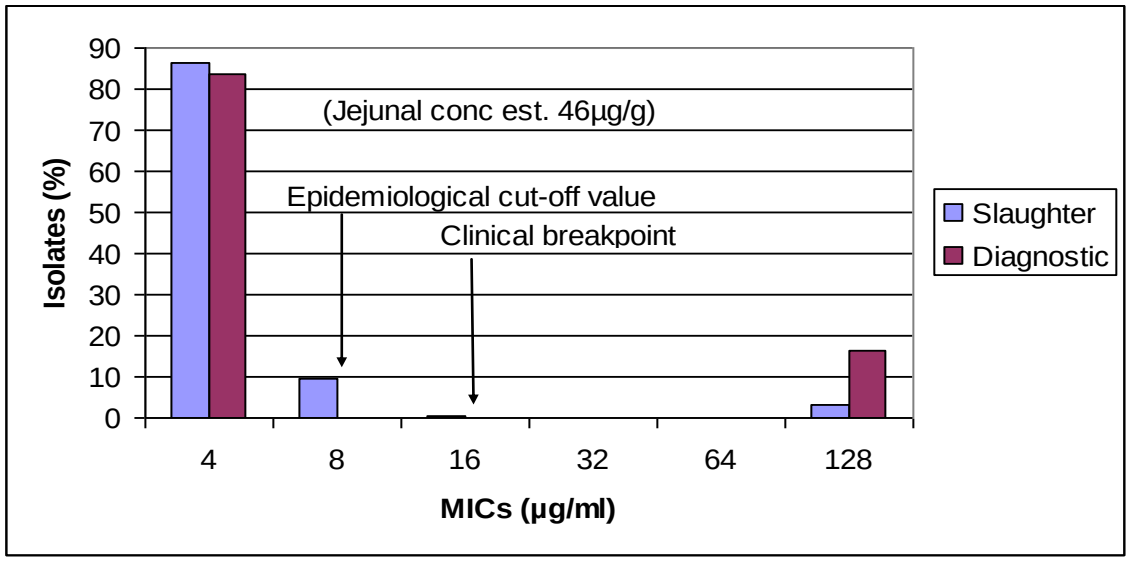
Neomycin is an aminoglycoside and is bactericidal and is concentration dependent. It is mainly gut active, but some drug is absorbed. Resistance is primarily plasmid induced and enzymes are produced, which alter the drug chemically and interfere with its binding to the 30S sub-unit of the ribosome. Reducing permeability of the bacterial cell is a less common means of inducing resistance. Transport across the cell membrane is an oxidative process and therefore aminoglycosides are not active in anaerobic conditions, such as exist in the large intestine.

Figure 3.17. Neomycin MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Apramycin

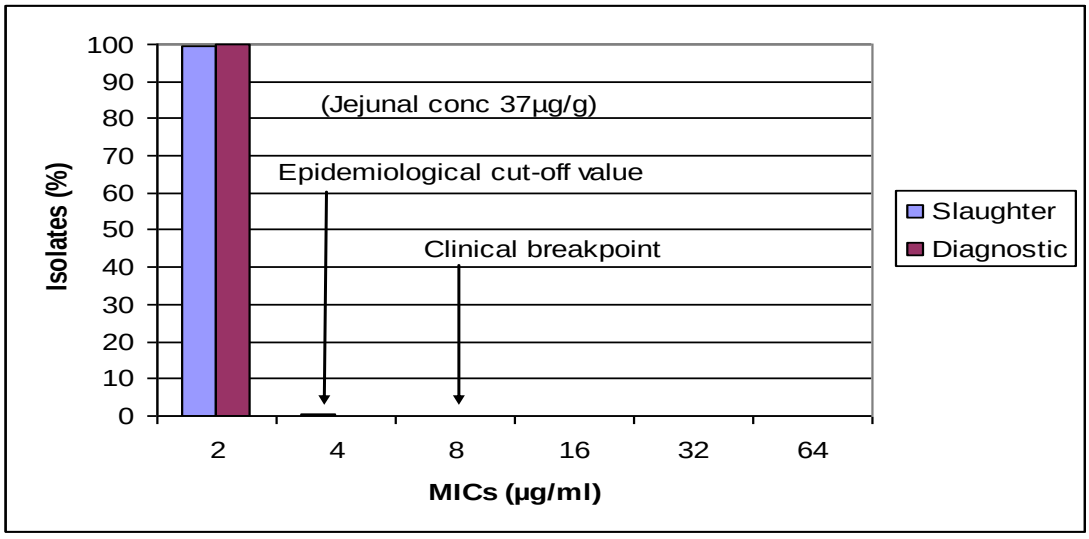
Figure 3.18. Apramycin MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Apramycin is also an aminoglycoside, which is more enzyme resistant to chemical change than neomycin. The same mechanisms of resistance do apply. Small quantities of the drug are absorbed but most pass through the intestines unchanged.

Colistin

Figure 3.19. Colistin MIC patterns against *E. coli* from porcine slaughter data (n = 208) and porcine diagnostic submissions (49) (Anon., 2005)



Colistin, also known as polymixin E, acts on the surface of bacteria and disrupts the structure of the cell membrane to increase cell permeability. It is primarily active against aerobic gram negative bacteria such as *E. coli*. Colistin is poorly absorbed from the

intestine. Resistance is rarely acquired, although some *Pseudomonas aeruginosa* strains have become resistant as a result of decreased bacterial permeability.

Table 3.5. Estimated antimicrobial pharmacokinetic relationship analysis for poorly absorbed products against their epidemiological cut-off values and clinical breakpoints (Analysis and calculations DB)

Antimicrobial	Feed level* (ppm)	Bioavail (%)	Faeces conc (µg/g)	Jejunal conc (µg/ml)	AUC 24h (µg.h/ml)	ECOV (µg/ml)	AUC/ECOV (h)	CBP (µg/ml)	AUC/CBP (h)
Spectinomycin	44	10	67	20	485	32	15	64	8
Neomycin	220	10	338	101	2435	4	609	8	304
Apramycin	100	10	153	46	1102	8	138	16	69
Colistin	66	10	125	37	888	4	222	8	111

*Based on 20ppm = dose of 1mg/kg liveweight per day

Spectinomycin concentrations approach an inhibitory effect at the AUC/ECOV level but AUC/CBP figure is low at 8h. The other three antibiotics reach likely bactericidal concentrations (AUC/ECOV = >100h). Neomycin and colistin also appear to be bactericidal using the AUC/CBP ratio, whereas apramycin is lower at 69h.

Conclusions

Clemens *et al* (1975) and Wiuff *et al* (2002) could have produced more accurate pharmacokinetic data in the small intestine if they had increased the number of sample points and looked earlier than 2h after administration. However, compensatory calculations can be made to overcome this.

Although there are limited published data on gut pharmacokinetics of antimicrobial drugs in the pig, the available data generally conform to PK/PD relationship values proposed in the literature for bactericidal compounds. These AUC/ECOV values correspond well to antimicrobial susceptibility patterns and also the AUC/CBP values, except for spectinomycin and enrofloxacin.

At this level, PK/PD relationships can only provide a guide to clinical outcome determination because of the nature of the double-dilution method of MIC and inter-laboratory variations, but increasing standardization will improve the chances of harmonisation. The susceptibility pattern data developed in this section plus the estimated PK values confirm what a powerful tool PK/PD analysis can be when applied to improving our understanding how drugs work in the gut. However, the level of sophistication and precision is probably not quite suitable for model applications at this stage.

3.4 Ileal infections – *Lawsonia intracellularis*

Introduction

Porcine proliferative enteropathy, commonly referred to as 'ileitis,' caused by *Lawsonia intracellularis*, is a common and widespread intestinal infection of pigs. In its acute form, it can cause mortality in finishing pigs and young breeding stock and in its more common chronic form, depression and unevenness of growth and poor FCE in growers and finishers.

The prevalence of *L. intracellularis* infection on farms in the United Kingdom and Ireland is high, at 94.9% (*Mortimer *et al*, 2000) and 62.2% of the finishing pigs tested were serologically positive, using a relatively sensitive immuno-fluorescent antibody test. This demonstrates that a large number of animals are exposed to the organism and may suffer from ileitis.

The characteristic lesions of epithelial cell proliferation and thickening of the ileal wall primarily affect the terminal ileum, but may extend into the caecum and proximal colon. Ileal lesions of over 1.5 meters length have been measured in artificial infection studies and the length of the lesion has been directly linked to growth rate depression (*Winkelman, 1999).

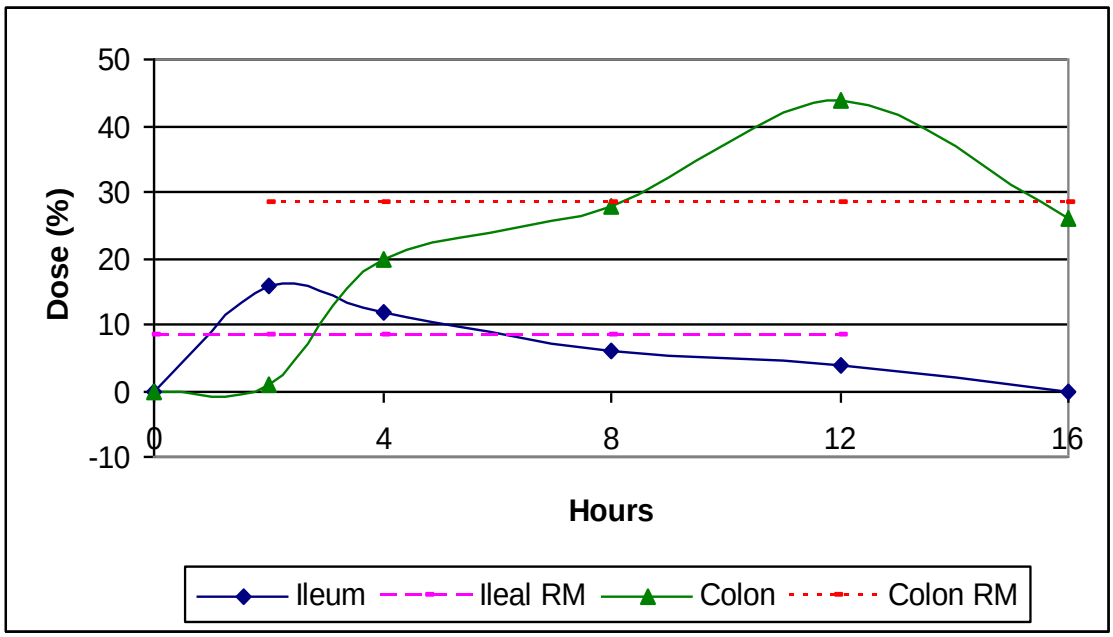
Several drug groups have been shown to be active against *L. intracellularis*, including the macrolides, tetracyclines, lincosamides and pleuromutilins (McOrist *et al*, 1995). These workers used a rat intestinal epithelial cell culture to determine intracellular minimum inhibitory concentrations (MICs). Many of the drugs in these groups now have therapeutic claims against this bacterium. However, with the introduction of the European Guideline (Anon., 2001a) for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances, there have been considerable uncertainties over the PK/PD relationships based on ileal contents concentrations (ICCs) and intracellular MICs on the one hand and clinical efficacy on the other. *Burch (2003) reviewed the PK/PD relationships for lincomycin and demonstrated that the ICC gave a good correlation with intracellular inhibition and clinical effect but not necessarily with the intracellular MIC. Recent work (Wattanaphansak *et al*, 2009) looked at the intracellular MICs of a variety of antimicrobial drugs over a wider MIC range against 10 US and EU isolates and a clearer correlation with PK and PD has emerged. This section examines the PK/PD relationships of antimicrobials against *L. intracellularis*.

Pharmacokinetics

Using the Clemens *et al* (1975) basic data and *Burch (2005b) intestinal model, the relationship between colonic contents or faecal concentrations of an antimicrobial substance can be correlated with the ileal contents concentration, the ileum being the major site of infection and lesion development.

The area under the curves represented by the concentration of the markers in the ileum and colon give an approximate relationship to the concentration of drugs e.g. antimicrobials, in these sections of the gut (Figure 3.20).

Figure 3.20. Percentage of dose present in the ileum and caecum at selected time points after administration and the calculated rolling mean (RM) figure over 12 h periods (Clemens *et al*, 1975; *Burch 2005b)



Taking the area under the curve over 0-12 h (AUC 12) for the ileum and the AUC for 2-14 h for the colon, the rolling means can be calculated and the concentration ratio of the ileal contents to the colon contents can be estimated (see Table 3.6).

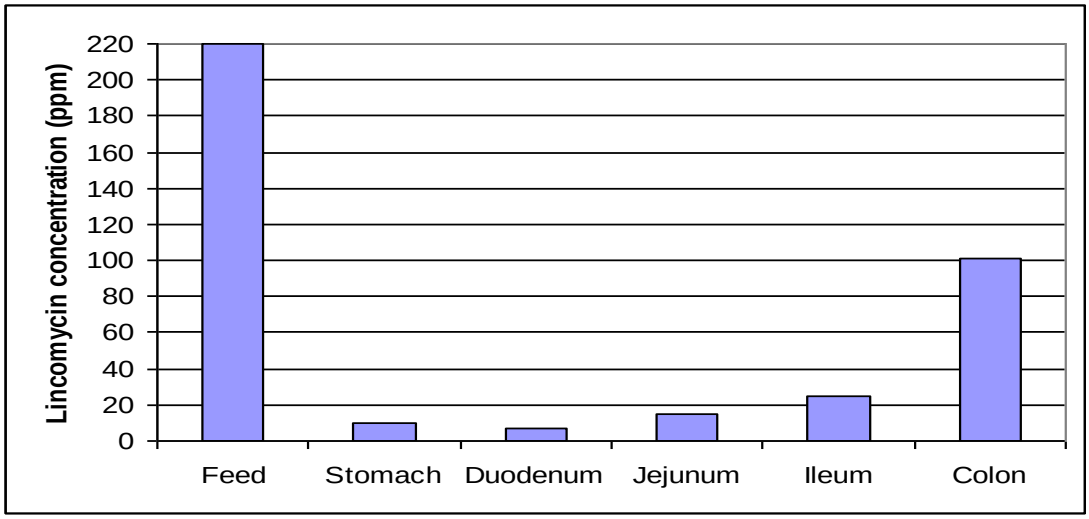
Table 3.6. Pharmacokinetic relationships between the colon and ileum using a marker substance (Calculations DB)

	Ileum (0-12 h)	Colon (2-14 h)
Area under the curve (AUC 12 h)	100	340
Rolling mean concentration 12 h	8.3	28.3
Ileal/colonic concentration ratio	0.294 : 1	-

There is approximately 29% of the drug concentration found in the ileum in comparison with the colon.

A relationship was reported by *DeGeeter *et al* (1980) following the administration of lincomycin at 220 ppm (approximate dose of 11mg/kg) in the feed over 23 days to pigs weighing on average 29.5kg (Figure 3.21). The ileal and colonic contents were 25.05µg/g and 101.01µg/g respectively giving approximately 25%. Concentrations in the other sections of the small intestine confirm the findings of Clemens *et al* (1975) that the transit is more rapid than in the colon, where contents concentrate as water is absorbed. A relatively steady state appears to exist after feeding for several days.

Figure 3.21. Concentrations of lincomycin in different regions of gut contents following administration in the feed at 220ppm for 23 days (*DeGeeter *et al,* 1980)



In this study, concentrations in the tissues of the different sections of the gut were also recorded and, in general, these were relatively low in comparison with the contents (Table 3.7).

Table 3.7. Comparative concentrations of lincomycin in various sections of the gut for both contents and gut wall tissues (*DeGeeter *et al,* 1980) (Calculations DB)

	Contents (ppm)	Tissue (ppm)	Tissue/contents ratio
Feed	220	220	1
Duodenum	7.2	1.4	0.19
Jejunum	14.5	1.3	0.09
Ileum	25.1	1.3	0.05
Colon	101	0.7	0.007

As few data were available on the drug concentrations in the ileum, *Burch (2005b) described or estimated the colonic contents concentrations for a number of antimicrobials, based on certain assumptions. Estimated data were based on the linear equivalent data for known concentrations to give the range. The mean of the range was used to give the concentrations usually for the lower in feed concentrations. The ileal contents concentration (ICC) was estimated by multiplying the colon contents concentration **by 0.29** (see Table 3.6).

Table 3.8. Estimated colonic contents concentrations (CCC) (µg/g) of various antibiotics at various in feed levels (ppm) (*Burch, 2005a & b)

Antibiotic/*concentration*	Levels in feed (ppm)/*Concentrations in colon (µg/g)*		
Valnemulin	200	75	25
Concentration	*5.2*	*1.68*	*0.56-0.65(0.61)E*
Tiamulin	220	110	44
Concentration	*8.05*	*2.84*	*1.12-1.61(1.37)E*

Antibiotic/*concentration*	Levels in feed (ppm)/*Concentrations in colon (µg/g)*		
Lincomycin	220	110	44
Concentration	*101*	*34.5*	*13.8-20.2(17.0)E*
Tylosin	200	100	40
Concentration	*68E*	*34E*	*14E*
Tylvalosin	200	100	50
Concentration	*>10-35(22.5)E*	*>5-17.5(11.3)E*	*>2.5-8.8(5.5)E*

E = estimate – proportionately related to reported values

Table 3.9. Estimated ileal contents concentrations (ICC) (µg/g) of various antibiotics at various in-feed levels (ppm) (colonic contents concentration (CCC) x 0.29) (*Burch, 2005b)

Antibiotic/ *concentration*	Levels in feed (ppm)/*Concentrations in ileum (µg/g)*		
Valnemulin	200	75	25
Concentration	*1.51*	*0.49*	*0.17- 0.19(0.18)E*
Tiamulin	220	110	44
Concentration	*2.33*	*0.82*	*0.32-0.47(0.40)E*
Lincomycin	220	110	44
Concentration	*29.29*	*10.01*	*4.0-5.86(4.93)E*
Tylosin	200	100	40
Concentration	*20E*	*10E*	*4E*
Tylvalosin	200	100	50
Concentration	*>2.9-10.15(6.53)E*	*>1.45-5.08(3.28)E*	*>0.73-2.55(1.60)E*

E = estimate – proportionately related to reported values

The pleuromutilins, valnemulin and tiamulin, attain relatively low concentrations in the ileum in comparison with lincomycin and tylosin; tylvalosin is in between (Anon., 2001b). Spectinomycin, in combination with lincomycin, is also approved for the treatment of ileitis at 44ppm of each product (88ppm combined). Spectinomycin is poorly absorbed from the gut and from a radio-labelled study (Anon., 2000), 79% remained in the gut, 4.5% was excreted via the urine and 0.05% found in the faeces, 12 hours post-dosing. An approximate concentration of the product can be calculated in the colon, e.g. 79-95.5% (say 90%) of the concentration in feed times 44ppm x 1.7 equals 67ppm. If this is then multiplied by 0.29, the estimated concentration in the ileum is approximately 19.5ppm or µg/g, for example.

Pharmacodynamics

McOrist *et al* (1995) investigated the extracellular and intracellular MICs of several drugs against a number of strains of *L. intracellularis*, using a monolayer cell culture of rat intestinal epithelial cells (IEC-18). The extracellular and intracellular MIC for each drug was determined when the inhibition was greater than 99%. The intracellular MIC for valnemulin was reported by *McOrist *et al* (1998). In an earlier paper, McOrist and Gebhart (1995) also described a minimum bactericidal concentration (MBC) test for a limited number of drugs against *L. intracellularis* (Table 3.10).

The extracellular and intracellular MICs were almost identical for most antibiotics. There was a large discrepancy between the MICs and MBC for tylosin, from 64 down to <4µg/ml respectively and also a reduction for tiamulin from 4 to <2µg/ml, yet the antibiotic exposure was shorter. Normally, the MIC is lower than the MBC, especially for macrolide and pleuromutilin antibiotics.

Table 3.10. Extracellular and intracellular MIC and MBC (>99% inhibition) for a number of strains of *L. intracellularis* (Mackie, 1996)

Antibiotic	No. of strains	Extracellular MIC (µg/ml)	Intracellular MIC (µg/ml)	No. of strains	Intracellular MBC (µg/ml)
Valnemulin	2	-	<2	-	-
Tiamulin	3	4	4	1	<2
Lincomycin	2	32	32	-	-
Tylosin	3	64	64	1	<4
Tilmicosin	2	2	2	-	-
Spectinomycin	1	32	32	-	-

Mackie (1996), one of the co-authors in McOrist *et al* (1995), described in her Master's Thesis the individual results of the strains tested. Not all strains were titrated to their lowest limits, so an actual MIC could not easily be determined for each isolate. Additionally, the assay method end point of determining a figure for a MIC could be considered an arbitrary microbiological standard (*Burch, 2003).

Table 3.11. Extracellular drug concentrations (EDC) and percentage inhibition of growth results to determine intracellular MIC (iMIC), *extracellular MIC* (*eMIC*) and iMBC for several drugs on various isolates of *L. intracellularis* (Mackie, 1996)

Drug	EDC (µg/ml) day 2-5	Strain 916/91 (NCTC12657)		Strain 1482/89 (NCTC12656)		Strain 51/89	
		iMIC	*eMIC*	iMIC	*eMIC*	iMIC	*eMIC*
Valnemulin	1 2 4 8	-	-	100	*100* *100* *100* *100*	- - 99.6 99.7	- - *99.6* *99.4*
Tiamulin	2 4 8	- - 99.4	- - *99.4*	96.4 100	*98.4* *100* *96.4*	- - 99.9	- - *99.4*
Lincomycin	0.25 1 4 16 32	98 80.5 80.5 28.6	*39.6* *7.1* *93.5* *93.5*	- - - 59.5 11.6	- - - *2.1* *23.8*	-	-
Tylosin	2 8 16	-	-	- - -	- - -	90.6 95 93.8	*74.8* *77.9* *96.8*

Drug	EDC (µg/ml) day 2-5	Strain 916/91 (NCTC12657)		Strain 1482/89 (NCTC12656)		Strain 51/89	
	32 100			- 99.7	- *99.7*	75 -	*90.6* -
Tilmicosin	0.125 0.5 2 8	- 90 90	- *90* *90*	-	-	100 98.5 99.2 98.5	*97.6* *97.6* *100* *100*
Spectinomycin	16 64	36 100	*100* *97.7*	-	-	-	-
Drug	EDC (µg/ml) day 2 only	Strain 916/91 (NCTC12657)		Strain 1482/89 (NCTC12656)		Strain LR189/5/83	
		iMBC		iMBC		iMBC	
Tiamulin	2	100		-		-	
Tylosin	2 4	-		99.4 100		100	

There are only minor differences between the eMIC and iMIC for valnemulin, tiamulin and tilmicosin. Lincomycin showed a different pattern between iMIC and eMIC with lower concentrations being less effective for the eMIC but higher levels were less inhibitory for the iMIC. This suggests a drug-related effect on the cell-culture system with a longer and higher exposure. Spectinomycin showed a lower inhibitory concentration was required for the eMIC, presumably because it has a more bactericidal activity against the unprotected organism before it enters the cell, as it has poor cell penetration. The internalization of the organism into the cell occurs in as little as 3 hours (*Gebhart, 2004) via membrane-bound vacuoles, before release into the cytoplasm.

Due to the limited drug concentrations that were studied, it was difficult to determine the precise intracellular MIC figures for the different drugs. However, Wattanaphansak *et al* (2009) carried out a study with 10 isolates of *L. intracellularis* from the US and EU, using McCoy cells instead of rat enterocytes and tested antimicrobial concentrations ranging from 0.125 to 128µg/ml. The antimicrobial drugs examined were carbadox (not EU), chlortetracycline, lincomycin, tylosin, tiamulin and valnemulin.

Table 3.12. Comparison of intracellular MICs for various antimicrobial drugs against 10 US and EU isolates of *L. intracellularis* (Wattanaphansak *et al,* 2009)

Antimicrobial drug	iMIC 50 (µg/ml)	iMIC 90 (µg/ml)	Range (µg/ml)
Carbadox	≤0.125	0.25	≤0.125-0.25
Chlortetracycline	8.0	64	0.125-64
Lincomycin	64	128	8.0-128
Tylosin	2.0	8.0	0.5-8.0
Tiamulin	≤0.125	≤0.125	≤0.125
Valnemulin	≤0.125	≤0.125	≤0.125

Carbadox, tiamulin and valnemulin all showed particularly low intracellular MICs in comparison with tylosin, lincomycin and chlortetracycline. From the susceptibility

patterns there are distinct bimodal patterns suggesting a reduction in susceptibility. This work appears to highlight some of the deficiencies in the original work by McOrist *et al* (1995).

Pharmacokinetic/pharmacodynamic relationships

Using the Wattanaphansak *et al* (2009) data a clearer picture emerges of the relationship between intracellular MIC and ileal contents concentration (ICC).

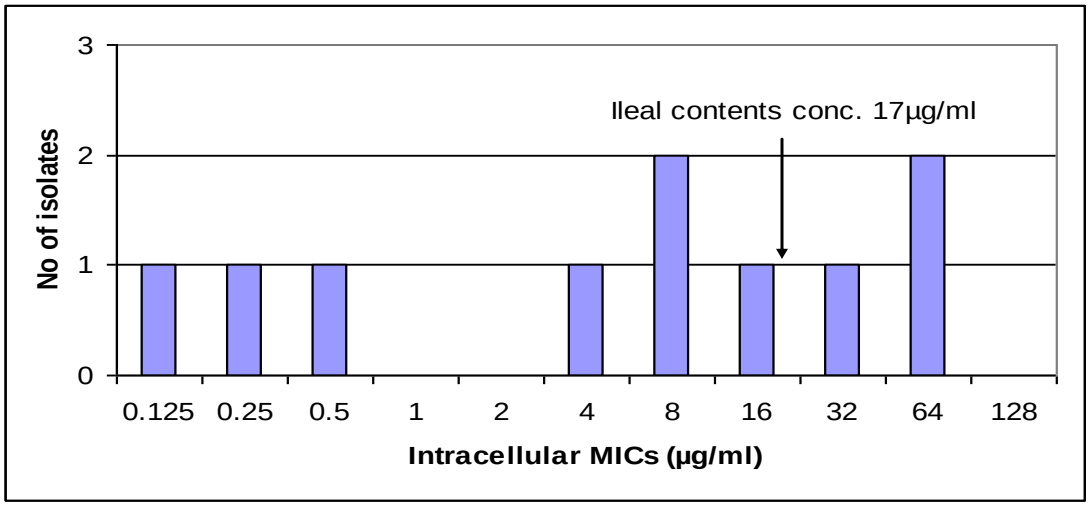
Table 3.13. Comparison of intracellular MICs for various antimicrobial drugs against 10 US and EU isolates of *L. intracellularis* and ileal contents concentration (ICC) (Wattanaphansak *et al,* 2009) (Analysis DB)

Antimicrobial drug	iMIC 90 (µg/ml)	ICC (µg/ml)	AUC (µg.h/ml)	AUC/iMIC 90 (h)
Carbadox 50ppm	0.25	-	-	-
Chlortetracycline 400ppm	64	17	408	6.4
Lincomycin 110ppm	128	10	240	1.9
Tylosin 100ppm	8.0	10	240	30
Tiamulin 150ppm	≤0.125	1.35	32.4	259
Valnemulin 75ppm	≤0.125	0.49	11.8	94

Only tiamulin and valnemulin are greater or around the AUC/MIC90 figure of 100-125h suggesting a treatment effect. Tylosin's figure is quite low but shows a likely inhibitory effect. Lincomycin's and chlortetracycline's AUC/MIC 90 figure is indicative of sub-inhibitory effects or actual resistance.

Chlortetracycline

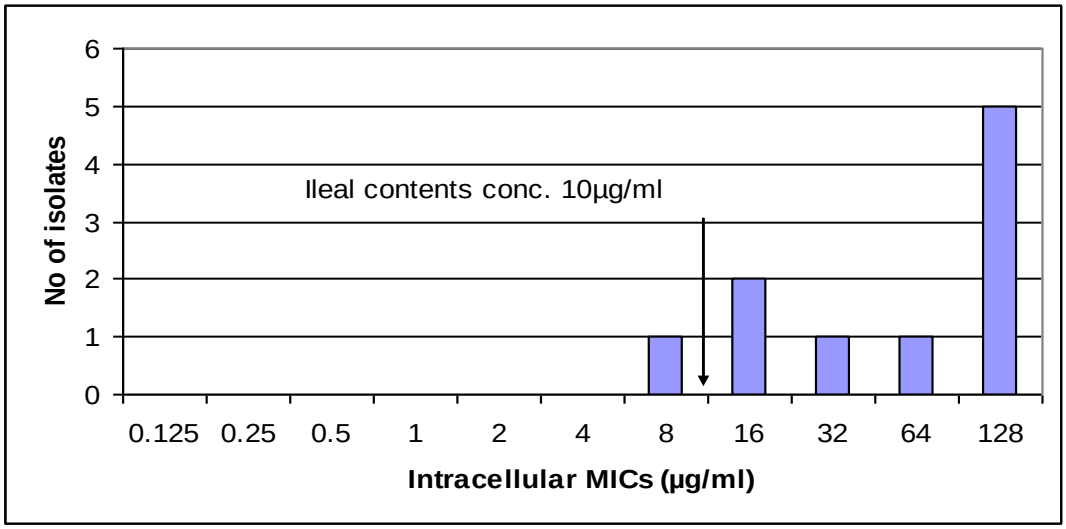
Figure 3.22. Susceptibility pattern for chlortetracycline iMICs against *L. intracellularis* and ileal contents concentration at 400ppm (Wattanaphansak *et al,* 2009)



There is quite a variation in the range of iMICs and there would appear to be a resistance pattern developing above 16µg/ml.

Lincomycin

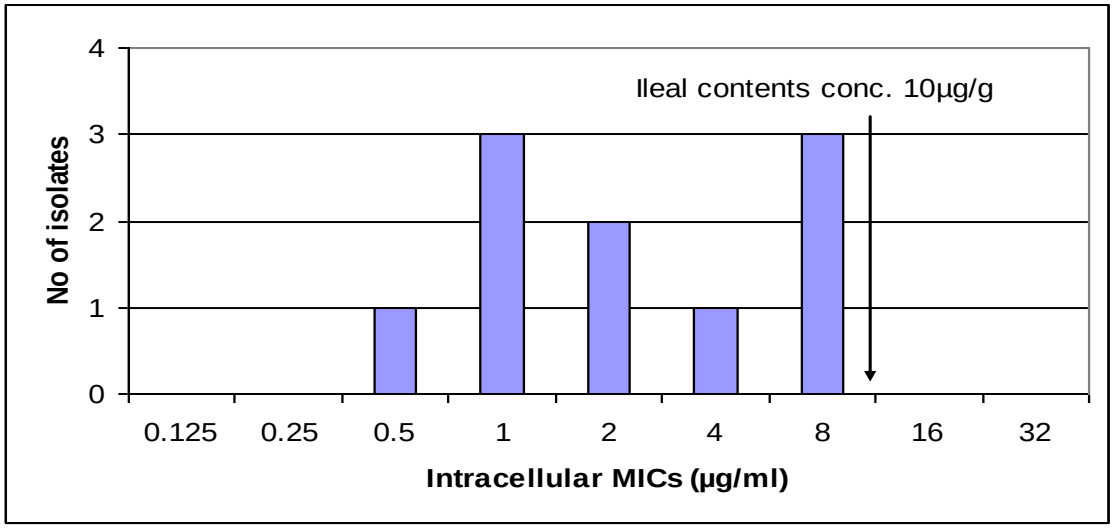
Figure 3.23. Susceptibility pattern for lincomycin iMICs against *L. intracellularis* and ileal contents concentration at 110ppm (Wattanaphansak *et al,* 2009)



From this work there would appear, that there may be quite a high level of resistance to Lincomycin. There is a bimodal susceptibility pattern, however.

Tylosin

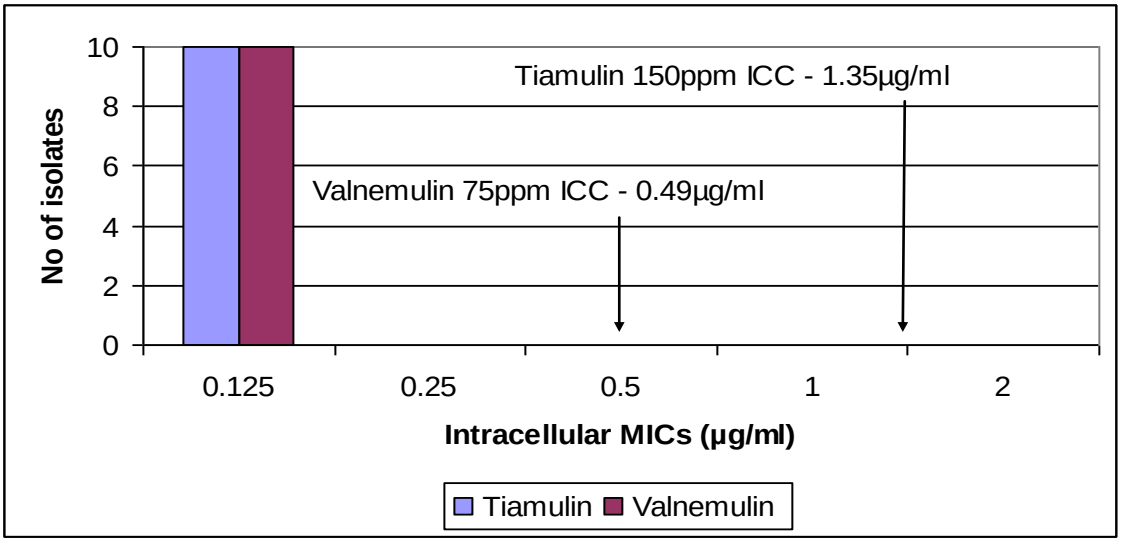
Figure 3.24. Susceptibility pattern for tylosin iMICs against *L. intracellularis* and ileal contents concentration at 100ppm (Wattanaphansak *et al,* 2009)



There appears to be a bi-modal susceptibility pattern but at the treatment level of 100ppm tylosin it appears that the ileal contents is above the iMIC range. Tylosin has been extensively used as a growth promoter up to 40ppm in many countries and the dip coincides with an ICC of 4µg/ml, which would be achieved at this level.

Tiamulin and valnemulin

Figure 3.25. Susceptibility pattern for tiamulin and valnemulin iMICs against *L. intracellularis* and ileal contents concentration at 150 and 75ppm respectively (Wattanaphansak *et al,* 2009)



The susceptibility pattern suggests that resistance strains have not yet developed to these antibiotics at this stage.

Clinical effects of various antibiotics on ileitis

Tiamulin

McOrist *et al* (1996) described an artificial infection challenge model and the activity of tiamulin at 50ppm in the feed for the prevention of ileitis and 150ppm for the treatment of ileitis. The pigs were infected with a cell culture infected with *L. intracellularis* (strain LR189/5/83.1) isolated from a 5-month old British pig with acute proliferative enteropathy and the infectious dose was calculated at 3.4 x 10^8 bacteria/pig. Tiamulin was administered at 50ppm in the feed, 2 days before infection and for a further 21 days until the trial was terminated. The treated group received tiamulin at 150ppm seven days post-challenge and was treated for a further 14 days when all of the pigs were necropsied. There was an infected, untreated control and an uninfected, untreated control group.

In the infected control, 6/7 pigs showed gross lesions and all showed histological lesions in the ileum and 5/7 in the caecum. Both the 50ppm tiamulin for prevention and 150ppm treated pigs showed no evidence of gross or histopathological lesions or the presence of

stained organisms. The intracellular concentration of tiamulin against this strain was ≤0.125µg/ml (Wattanaphansak *et al*, 2009) and the estimated intestinal contents concentration was approximately 1.35µg/ml for 150ppm and 0.45µg/ml for 50ppm. The AUC/MIC was 259h and 86h respectively and both the 150ppm and 50ppm were completely effective for treatment and prevention.

Tylosin

McOrist *et al* (1997) carried out a similar trial with tylosin, using the same infected cell culture with *L. intracellularis* (strain LR189/5/83.1). Tylosin was given preventatively in the feed at 40ppm and 100ppm, four days prior to infection and for a further 16 days after infection when the levels were dropped to 20 and 40ppm, respectively, for the remaining 12 days. A further group was treated seven days post-infection for 21 days with tylosin at 100ppm, when the trial was terminated and the pigs autopsied. None of the pigs on the tylosin prevention or treatment programmes showed any gross or histopathological signs of infection with *L. intracellularis.* The infected, untreated control showed gross lesions in 5/8 pigs and histopathological lesions in 7/8 in the ileum and 3/8 in the caecum. It can be concluded that tylosin at 40/20ppm and 100/40ppm were highly effective in the prevention of ileitis and tylosin at 100ppm was effective in the treatment of ileitis. The iMIC for this particular strain was 1.0µg/ml (Wattanaphansak *et al*, 2009). The ileal contents concentrations were estimated to be 4 and 10µg/ml for 40 and 100ppm tylosin, respectively, and the AUC/MIC figures were 96 and 240h. These figures are quite high but confirm the likely efficacy of tylosin for both prevention and treatment at these levels against a sensitive isolate of *L. intracellularis*. All of the treated groups showed improved performance over the untreated and uninfected control group and especially over the untreated infected control.

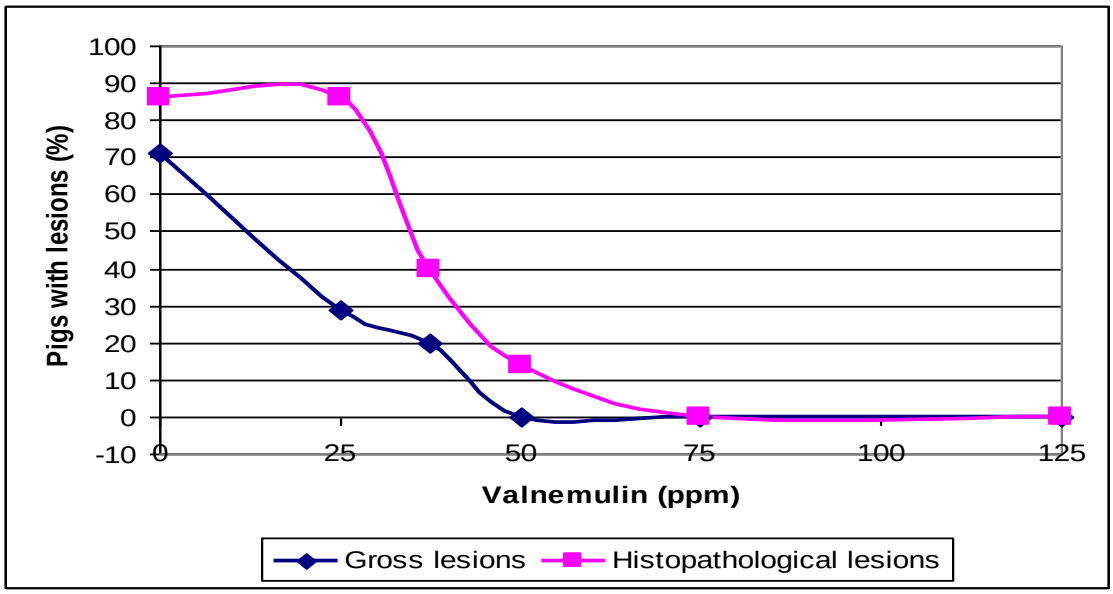
Valnemulin

In a third artificial challenge study, *McOrist *et al* (1998) looked at a range of doses of valnemulin in feed for the prevention and treatment of ileitis. The same challenge strain of *L. intracellularis* (strain LR189/5/83.1) was used. The medication for prevention at 25, 37.5 and 50ppm started two days before challenge and continued for a further 21 days. Treatment was administered seven days post- challenge for 14 days when the trial was terminated and the pigs necropsied. The results are summarised in Table 3.14.

Table 3.14. Dose-titration study to evaluate valnemulin for the prevention and treatment of an artificial challenge with *L. intracellularis* (*McOrist *et al,* 1998)

Valnemulin (ppm)	No. pigs gross lesions	No. pigs histopathological lesions	Histopathological lesion score (%)
0	5/7	6/7	100
25 (prevention)	2/7	6/7	46
37.5 (prevention)	1/5	2/5	22
50 (prevention)	0/7	1/7	9
75 (treatment)	0/7	0/7	0
125 (treatment)	0/7	0/7	0

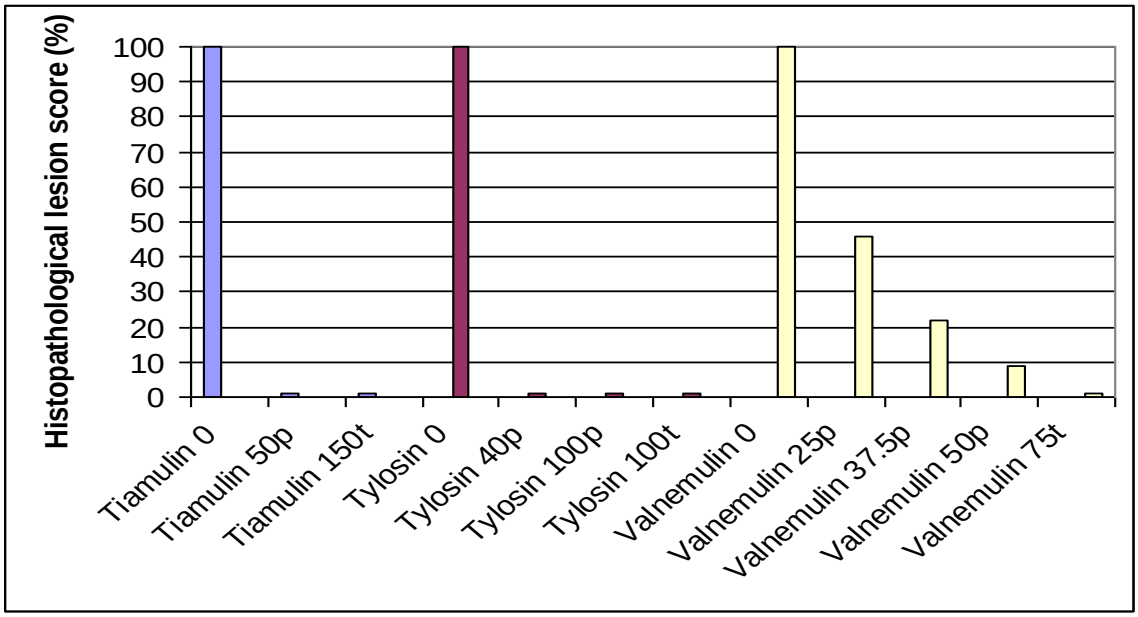
Figure 3.26. Effect of valnemulin for prevention and treatment on gross and histopathological lesions of ileitis (*McOrist *et al,* 1998)



There was a dose/effect with valnemulin between 25-50ppm for the prevention of ileitis. Gross lesions were not seen at 50ppm, but histopathological lesions could be seen in 1/7 pigs. Both treatment levels of 75 and 125ppm eliminated histopathological lesions of *L. intracellularis*. The iMIC for valnemulin was ≤0.125µg/ml (Wattanaphansak *et al*, 2009) and the estimated intestinal contents concentrations were 0.49µg/ml for 75ppm and 0.18 µg/ml for 25ppm valnemulin. The AUC/iMIC for 25ppm was 35h, which is indicative of an inhibitory effect but at 75ppm it was 94h which is approaching an expected bactericidal concentration or treatment effect.

In a comparison of three studies with tiamulin, tylosin and valnemulin, using the McOrist model and same challenge strain of *L. intracellularis* an interesting disease control pattern is demonstrated. Tiamulin and tylosin were completely effective at both their preventive and treatment levels but there is a definite dose-titration pattern down to 75ppm for valnemulin.

Figure 3.27. Comparative efficacy of various antimicrobials in the prevention (p) and treatment (t) of ileitis using the McOrist challenge model (Comparison DB)



Using a different artificial challenge model, *Winkelman *et al* (2000a) also looked at the effect of valnemulin at 25 and 50ppm in the prevention of ileitis. In this model, an *L. intracellularis* infected mucosal homogenate from the intestines of previously infected pigs was used. Each pig received an approximate challenge dose of 1 x 10^9 organisms. Medication was started at the time of challenge and continued for 21 days when the trial was terminated and the pigs necropsied. This model is considered a more severe challenge than the McOrist model, with occasionally high levels of mortality being induced in the untreated controls. They also use lesion length as a parameter, which reflects the more severe gross lesions observed. Larger numbers of pigs are able to be used - in this case, 110 pigs.

Table 3.15. Efficacy of valnemulin for the prevention of ileitis (*Winkelman *et al,* 2000a)

Treatment group (ppm)	Mortality (%)	Intestinal lesion length (cm)
Valnemulin 0	6.7	141.3 (-)
Valnemulin 25	2.5	94.6 (-33%)*
Valnemulin 50	0	72.0 (-49%)*

* p= <0.05

Gross lesion production and mortality is reduced by valnemulin, as the dose increases to 50ppm which gave the better results. In comparison with the McOrist model, where gross lesions were eliminated by 50ppm valnemulin, in the Winkelman model, they were reduced by only 49%. Unfortunately, the strain of *L. intracellularis* used in the model could not be grown in cell culture (C. Gebhart – personal communication) and therefore the iMICs have not been determined.

In a similar study (*Winkelman *et al*, 2000b) valnemulin was included in feed at 0, 25, 37.5 and 50ppm and administered for 5 days before infection and for a further 21 days. Tylosin at 110ppm was included as a positive control. The mortality was 32% in the negative controls and zero in the valnemulin and tylosin treated groups. Unfortunately, the lesion lengths were not reported.

Lincomycin

*Winkelman (1999) reported on a study with lincomycin, using the same infectious challenge model, but also injecting pigs intramuscularly with prednisolone acetate at the start of infection to enhance the onset of the disease. Pigs were medicated for four days prior to infection and for a further 31 days with lincomycin at 0, 44 and 110ppm and tylosin at 110ppm as a control. The trial was terminated and the pigs autopsied on day 35.

Table 3.16. Efficacy of lincomycin and tylosin for the prevention of ileitis (*Winkelman, 1999)

Treatment group (ppm)	Mortality (%)	Intestinal lesion length (cm)
Lincomycin 0	52	160 (-)
Lincomycin 44	4	84 (-48%)*
Lincomycin 110	12	79 (-51%)*
Tylosin 110	16	109 (-32%)

* p= <0.05

The mortality rate was very high in the untreated controls, demonstrating the severity of the challenge model. The lesion length was also long in the controls, but they were significantly reduced by lincomycin at 44 and 110ppm, but, surprisingly, there was little dose-related difference between the two groups. Both were better than the tylosin 110ppm control.

Tylvalosin (formerly Acetylisovaleryltylosin)

Tylvalosin was investigated by *Winkelman & Tasker (2002) for the treatment of ileitis. Some minor changes were made to the model; the infectious challenge dose was given on two consecutive days so each pig received approximately 2.8 x 10^9 organisms. Medication with tylvalosin at 0, 50 and 100ppm was given for 10 days, seven days after the challenge. Tylosin at 100ppm was administered as the positive control for 21 days and all of the pigs were necropsied.

Table 3.17. Efficacy of tylvalosin and tylosin for the treatment of ileitis (*Winkelman and Tasker, 2002)

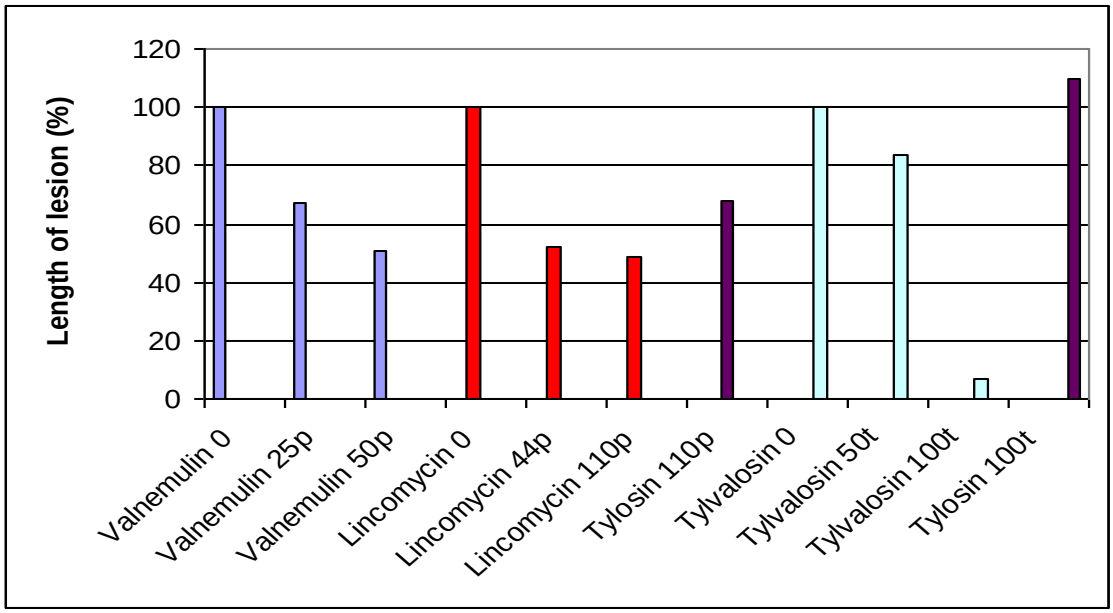
Treatment group (ppm)	Mortality (%)	Intestinal lesion length (cm)
Tylvalosin 0	15	109.5 (-)
Tylvalosin 50	13.3	91.9 (-16%)
Tylvalosin 100	0	8.0 (-93%)*
Tylosin 100	16.7	120.1 (+10%)

* p= <0.001

In this study, with a heavy infectious challenge, tylosin at 100ppm performed less well than the untreated controls by the parameters of mortality and lesion length, which is surprising. Tylvalosin at 50ppm showed only modest improvements over the untreated controls, but at 100ppm there was no mortality and lesion length was reduced by 93%, which is exceptional using the Winkelman challenge model. In addition, the length of medication was half that of tylosin (10 and 21 days respectively) so a strong bactericidal effect can be anticipated at this level in the feed, suggesting the iMIC was comparatively low <4.0µg/ml.

The strain of *L. intracellularis* used in this model may not be as susceptible to tylosin as the one used in the McOrist model.

Figure 3.28. Comparative efficacy of various antibiotics in the prevention (p) and treatment (t) of ileitis using the Winkelman challenge model (Comparison DB)



From this work, valnemulin at 50ppm is approximately equivalent to lincomycin at 44ppm, but superior to tylosin at 100ppm for prevention of ileitis. Tylvalosin at 100ppm was outstanding in comparison with tylosin 100ppm and potentially the other compounds for the treatment of ileitis.

Discussion and Conclusions

With the increasing requirement to use PK/PD relationships and modelling, as supporting evidence for product approval in the EU, it is useful to determine some of the baselines for their use for individual diseases and with individual antibiotics. From this work, a PK relationship can be established between colon contents and ileal contents concentration, where little published information is available.

Using PK/PD relationships has also highlighted some disparities in the original pharmacodynamic data (McOrist *et al*, 1995) such as the iMIC for tylosin at 64µg/ml, when ileal concentrations did not reach anywhere near it. It is probably the most commonly used product for the treatment and prevention of ileitis but the recent work by Wattanaphansak *et al* (2009) has really explained the discrepancies.

In fact, the intracellular MIC determinations were highly effective models in predicting the likely efficacy of antibiotics to inhibit or treat *L. intracellularis* infection (McOrist *et al*, 1995 and Wattanaphansak *et al*, 2009) although the latter's work has proven more useful for PK/PD relationship determination.

From a clinical view point, the two main models used, do give substantially different results. The McOrist model, using the infected cell culture, is more sensitive and therefore correlations between PK/PD are more easily determined. The Winkelman model, using ground up, infected intestine, is cruder but different endpoints can be used to determine treatment effects. Unfortunately, as the MIC for the isolate cannot be determined, more precise PK/PD correlations cannot be calculated. Nevertheless, tylvalosin certainly demonstrated a substantial therapeutic effect at 100ppm in the feed in comparison with other antimicrobials tested and tylosin, in particular.

3.5 Colonic bacterial infections – *Brachyspira hyodysenteriae* and *B. pilosicoli*

Introduction

Swine dysentery caused by *B. hyodysenteriae* is one of the most important enteric infections in pigs primarily affecting the large intestine or colon. It can cause diarrhoea, leading to dysentery, and ultimately death. The use of antimicrobial drugs has been the most important way of controlling the infection over many years in infected farms. Vaccines have not been developed commercially due to poor reliability and cross protection. Eradication programmes have been developed and implemented with some success. Some data regarding concentrations of drugs in the colon or faeces is available and there is increasing MIC data for *B. hyodysenteriae*, so the PK/PD relationships can be explored. Porcine spirochaetal diarrhoea or 'colitis', caused by *B. pilosicoli*, is generally a milder condition causing a moderate degree of diarrhoea and mucus production but very rarely an increased mortality. The organism occupies the same sites as *B. hyodysenteriae*, such as the surface and crypts of the colonic mucosa but generally is less invasive.

It is the purpose of this section to look at the antibiotics valnemulin, tiamulin, lincomycin, tylosin and tylvalosin and to try to correlate their PK/PD relationships with their clinical activity, to help determine suitable clinical breakpoint relationships with their MICs or MBCs.

Colonic contents concentrations of various antibiotics

Colonic contents concentrations (CCCs) of various antimicrobials are not always publicly available and sometimes have to be estimated. In general terms, products that

are well absorbed and metabolised in the liver tend to have low concentrations in the colon. Products that are poorly absorbed have higher concentrations, but these may also be subjected to digestive action and metabolic breakdown in the intestines. Products that are not absorbed concentrate in the large intestine and, commonly, the increase in concentration from food to faeces is 1.7 times (O'Callaghan *et al*, 1971).

Valnemulin and tiamulin are well absorbed (greater than 95%), extensively metabolised and excreted by the bile and their gut concentrations are relatively low (valnemulin, Econor, product data; tiamulin, *Anderson *et al*, 1994). Lincomycin is moderately absorbed, metabolised in the liver and excreted by the bile and kidney and achieves moderate levels in the colon (*DeGeeter *et al*, 1980). Tylosin, a mixture of antibiotics with factor A being the most significant, is relatively poorly absorbed (22.5% bioavailable) (Anon., 1997). It is metabolised and excreted mainly by the liver, but the product is also broken down in the intestine to a large degree and the activity is primarily due to bio-active metabolites such as relomycin (tylosin D) and dihydrodesmycosin in the faeces. The relative 'tylosin-like' activity can be estimated at 20% of the original dose, multiplied by 1.7, the concentration effect of feed to faeces based on O'Callaghan et al (1971) to give the final colonic concentration. Tylvalosin, a re-fermented product derived from tylosin, has been registered in the European Union for the treatment of swine dysentery. There is no published data on its CCCs, although it has been extensively metabolised, so that approximately 2-7% of the radio-labelled dose found in the faeces was parent compound (Anon., 2001b) and the rest were metabolites, which may have some antimicrobial activity, but they are not specified.

Table 3.18. Colonic contents concentrations (µg/g) of various antibiotics at various in feed levels (ppm) (*Burch 2005a)

Antibiotic/concentration	Levels in feed (ppm)/Concentrations in colon (µg/g)		
Valnemulin	200	75	25
Concentration	*5.2*	*1.68*	*0.56-0.65(0.61)E*
Tiamulin	220	110	44
Concentration	*8.05*	*2.84*	*1.12-1.61(1.37)E*
Lincomycin	220	110	44
Concentration	*101*	*34.5*	*13.8-20.2(17.0)E*
Tylosin	200	100	40
Concentration	*68E*	*34E*	*14E*
Tylvalosin	200	100	50
Concentration	*>10-35(22.5)E*	*>5-17.5(11.3)E*	*>2.5-8.8(5.5)E*

E – estimate – proportionately related to reported values.

From this data, it can be observed that there is a fairly linear relationship between the concentration given in the food and the concentration achieved in the colon contents. This means that it is difficult to set a simple breakpoint as there is often a 2.5-5-fold difference between a prevention level and a treatment level given in feed and, consequently, the antibiotic concentration achieved in the colon. The concentration in feed should be specified.

Pharmacodynamics of various antibiotics against *B. hyodysenteriae* and *B. pilosicoli*

There are a number of difficulties concerning the pharmacodynamics of the antibiotics against both *B. hyodysenteriae* and *B. pilosicoli*. There are three basic methods of growing the organisms and determining their MICs or MBCs and each one can give slightly different results, which may affect any precision.

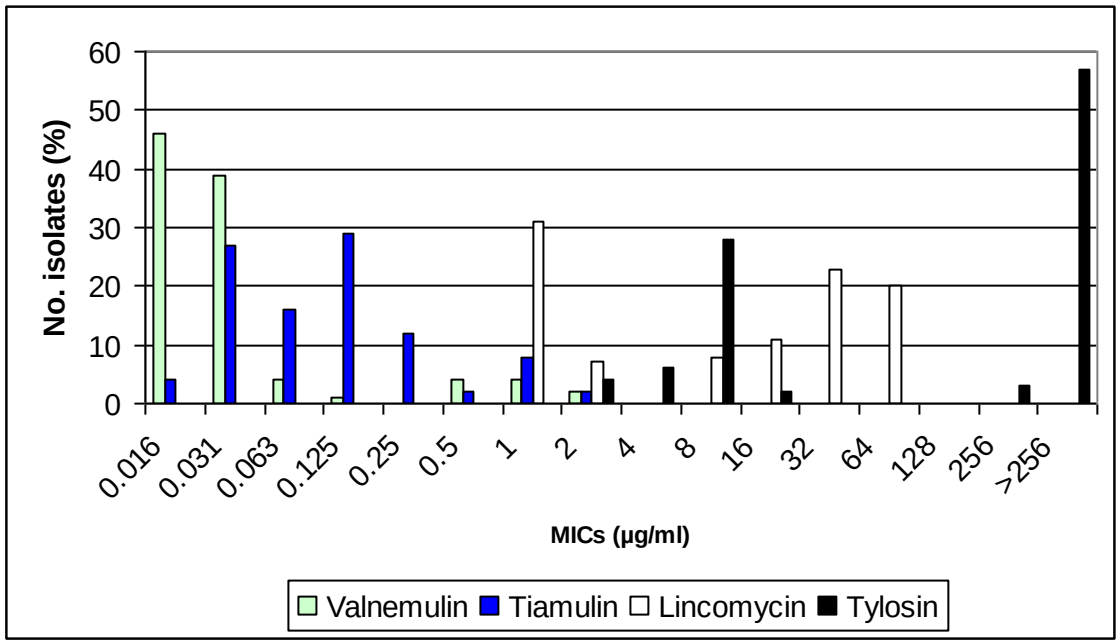
The three common methods for culture are broth, micro-broth plates and agar plates. In the broth culture, the organism is grown in doubling dilutions of antibiotic solution. The MIC is determined by the lowest concentration where the organism does not grow. The MBC is determined by sub-culturing the broth, where there is no growth into fresh broth without an antibiotic, or onto blood agar plates and seeing if the organism will grow up once the antibiotic has been diluted. The MBC is the lowest concentration of antibiotic where no growth occurs on sub-culture. Buller and Hampson (1994) demonstrated on 30 isolates of *B. hyodysenteriae* that the **MBC/MIC ratio** for tiamulin was, on average, 2.2 (range 1-4), lincomycin 1.7 (range 1-4) and tylosin 1.4 (range 1-4). This is a surprisingly small difference, considering the antibiotics are considered as primarily bacteriostatic. The micro-broth technique is similar to the broth technique, but uses microtitre plates. The blood agar plate test again uses doubling dilutions of the antibiotic and the plate with the lowest concentration of antibiotic, where there is no growth is the MIC. This test is probably the most widely used, especially in the UK. Interestingly, it has been observed (J. R. Thomson - personal communication) that there were usually no live organisms at the site of inoculation on the agar plate test, suggesting that they have been killed and that the plate test is possibly giving an effective MBC rather than an MIC. When the MICs from micro-broth or agar plate tests were compared (Rohde *et al*, 2004) in 221 strains in Germany, it was found that there was, on average, a one dilution difference, with the micro-broth technique giving the lower result for both tiamulin (range -2 to +5) and valnemulin (range -1 to +4). This is equivalent to a MBC/MIC ratio of two, which was observed by Buller and Hampson (1994) for tiamulin.

Karlsson *et al* (2002) reviewed the comparative activity (MICs) of valnemulin, tiamulin, lincomycin and tylosin against 76 field isolates of *B. hyodysenteriae* in Australia, using a micro-broth double-dilution technique. It was rare at that time to have such a large number of isolates in one report and this gives a good overview of the relative susceptibilities to the antibiotics and their sensitivity patterns.

Table 3.19. *In-vitro* activity of various antibiotics against 76 Australian field isolates of *B. hyodysenteriae* (Karlsson *et al,* 2002)

Antibiotic	MIC 50% (μg/ml)	MIC 90% (μg/ml)	Range (μg/ml)
Valnemulin	0.031	0.5	≤0.016 – 2.0
Tiamulin	0.125	1.0	≤0.016 – 2.0
Lincomycin	16	64	≤1.0 - 64
Tylosin	>256	>256	≤2.0 - >256

Figure 3.29. *In-vitro* activity of various antibiotics against 76 Australian field isolates of *B. hyodysenteriae* (Karlsson *et al*, 2002)



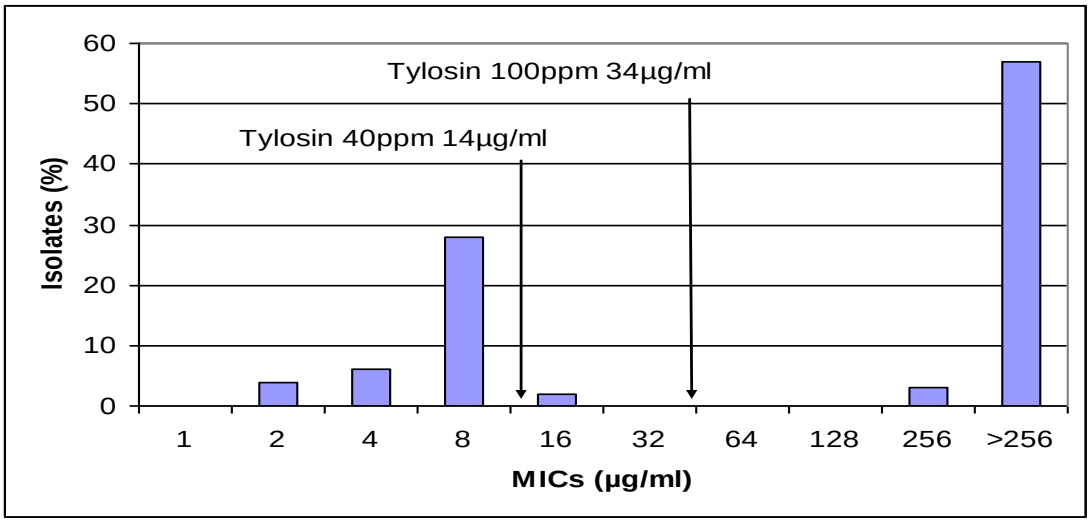
The MICs are generally lower for the pleuromutilin antibiotics, valnemulin and tiamulin, in comparison with lincomycin and tylosin. Higher gut concentrations are achieved with tylosin and lincomycin but in addition there would appear to be an extensive resistance pattern associated with tylosin.

Tylosin

If compared with the colonic contents concentrations (CCCs) of the antibiotics, it is only the tylosin isolates that are greatly in excess e.g. MICs ≥ 256µg/ml in comparison with a CCC maximum of 34-68µg/g. This suggests true resistance and is reflected in the tylosin pattern of MICs, as there is a large gap between the MIC of 16 to 256µg/ml.

Karlsson *et al* (2002) postulated that at the dips, mutations had taken place in the bacteria, changing their antimicrobial susceptibility. In the case of tylosin, it is a major leap leading to complete resistance, but it is not quite so clear for the other antibiotics.

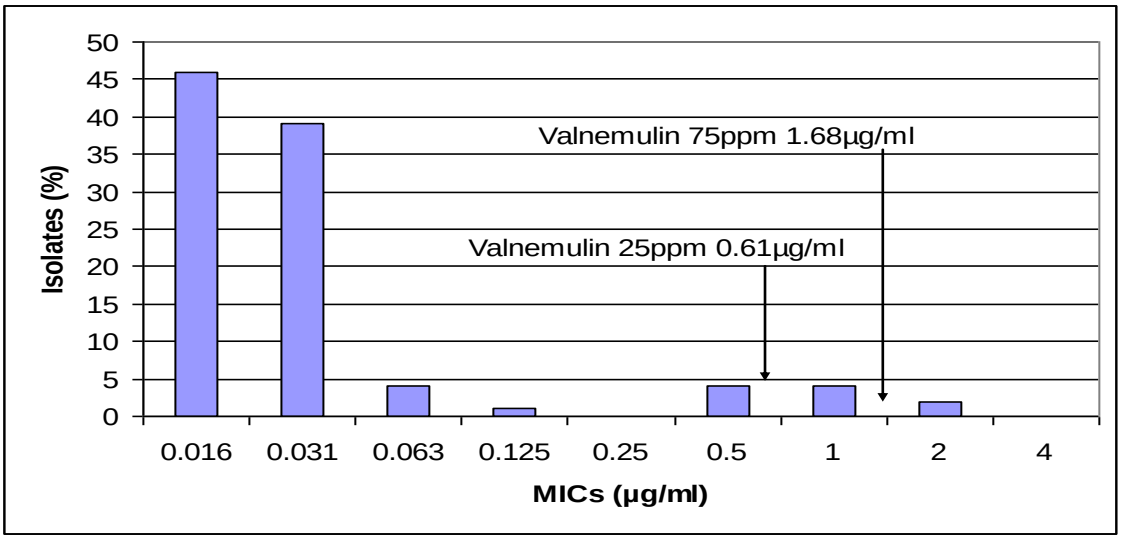
Figure 3.30. Susceptibility pattern of 76 isolates of *B. hyodysenteriae* against tylosin and its colon contents concentrations at various in-feed inclusions (Karlsson *et al,* 2002)



Valnemulin

Valnemulin by comparison, demonstrated a bi-modal susceptibility pattern with a wild type cut-off point at 0.125µg/ml. There appears to be a cluster of first-step mutants, which may not be completely controlled by 75ppm valnemulin in feed. The mutations are thought to be changes associated with the binding sites on the 23S sub-unit of the main 50S sub-unit of the ribosome (Pringle *et al*, 2004)

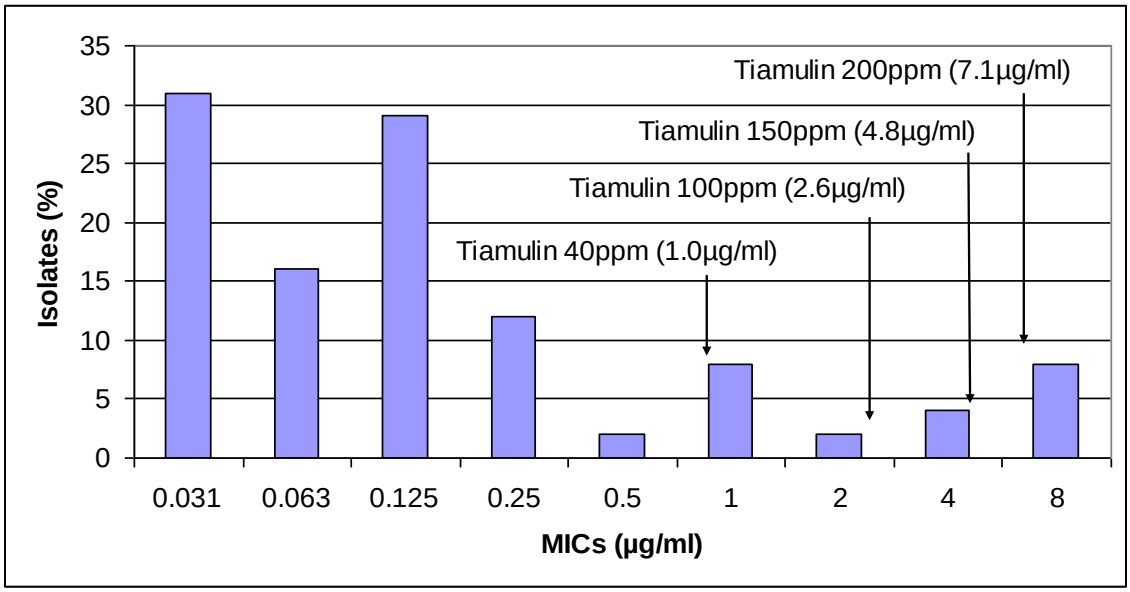
Figure 3.31. Susceptibility pattern of 76 isolates of *B. hyodysenteriae* against valnemulin and its colon contents concentrations at various in-feed inclusions (Karlsson *et al,* 2002)



Tiamulin

Tiamulin has a mainly tri-modal pattern with the main change at 0.5µg/ml, its wild type cut-off value or ECOV. There are two step mutations initially up to 2.0 µg/ml and then secondly >2.0µg/ml.

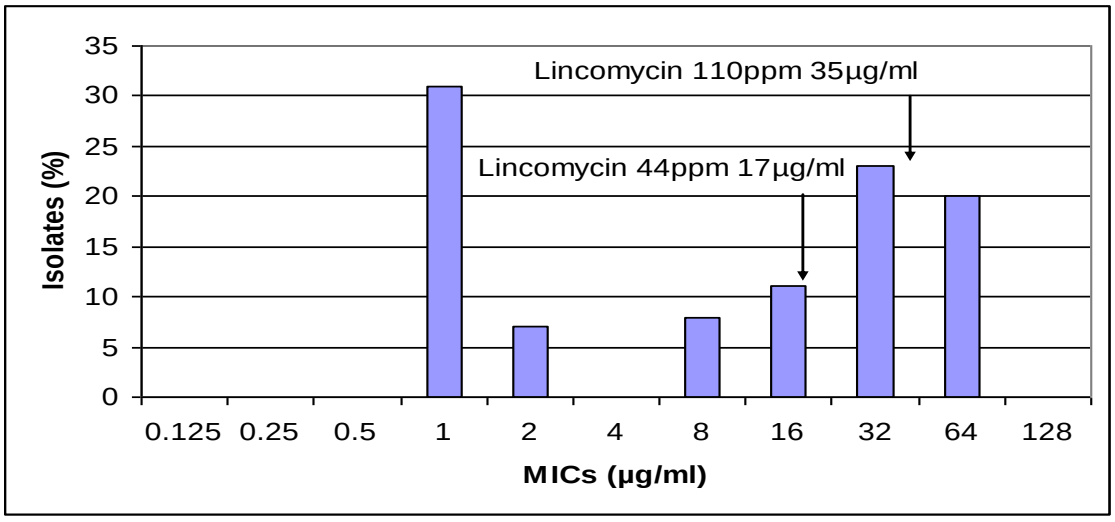
Figure 3.32. Susceptibility pattern of 76 isolates of *B. hyodysenteriae* against tiamulin and its colon contents concentrations at various in-feed inclusions (Karlsson *et al,* 2002; Karlsson *et al,* 2004)



Lincomycin

Lincomycin has a bimodal pattern with a gap at 4.0µg/ml and a second wave between 8-64µg/ml. This, in relation to its colon contents concentration, possibly would not identify true resistance but reduced susceptibility or increasing tolerance to the antibiotic at 110ppm but susceptibility at 220ppm, where the CCC was recorded at 101µg/ml (*DeGeeter *et al*, 1980).

Figure 3.33. Susceptibility pattern of 76 isolates of *B. hyodysenteriae* against lincomycin and its colon contents concentrations at various in-feed inclusions (Karlsson *et al,* 2002)

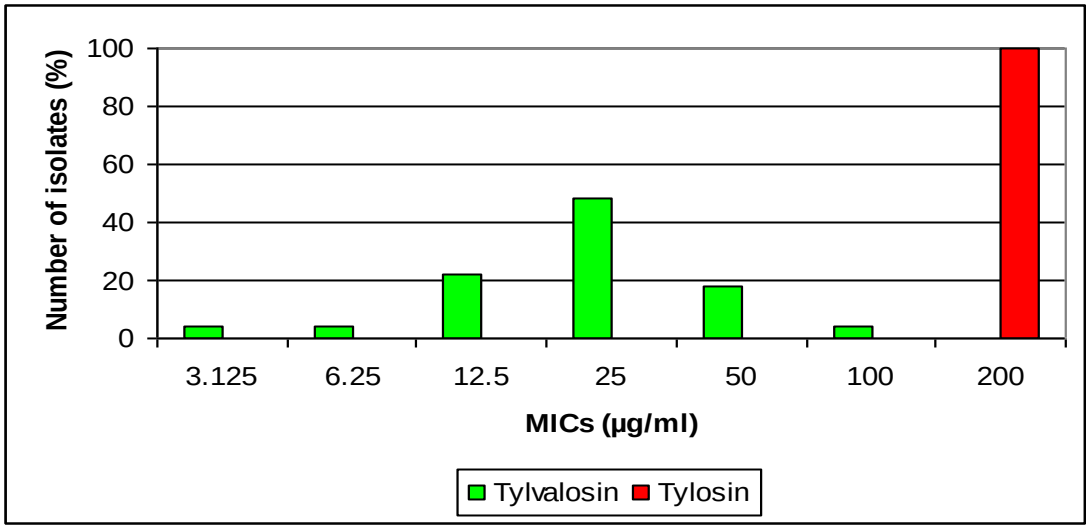


The driver for these patterns is thought to be the use of antimicrobials. There is a natural range of susceptibility within a microbial population (wild type population), but with products such as tiamulin, lincomycin and tylosin, which have been used in pigs for over 30 years, an antibiotic use pattern has developed on the natural pattern. The induction of resistance or MIC increase is characteristic for each pathogen/antimicrobial combination and the 'mutant selection window' (Drlica, 2003) starts at approximately the wild type breakpoint MIC or ECOV and above, to the level of the next mutation. Mutant selection is usually a slow and step-wise development, but sometimes there is a major jump, as in the case of tylosin. In *in-vitro* resistance development studies, the peri-MIC levels are sub-cultured and used to select for resistance. Therefore, from the above MIC study, the patterns are important to help demonstrate what are the likely active bio-concentrations achieved by the antibiotics to have selected for the mutation.

Tylvalosin

Tylvalosin demonstrates a clearly different sensitivity pattern from the parent compound tylosin (*Cizek *et al,* 2003) using an agar plate double dilution test on 50 Czech field isolates of *B. hyodysenteriae*. This is thought to be associated with the additional side chain attached to the tylosin molecule, which enhances its attachment at the 50S RNA subunit.

Figure 3.34. Susceptibility of 50 Czech *B. hyodysenteriae* isolates to tylvalosin and tylosin (agar plate test) (*Cizek *et al,* 2003)



Interestingly, they showed a uni-modal curve for tylvalosin and complete resistance to tylosin. They also commented that MIC values of 12.5-25µg/ml corresponded to clinical efficacy, which would give a possible susceptibility of 78% of isolates.

Brachyspira pilosicoli

Similar MIC results against *B. hyodysenteriae* are demonstrated by *B. pilosicoli* (*Kinyon *et al*, 2002) on 25 US isolates for valnemulin, tiamulin, lincomycin and tylosin, using an agar plate test.

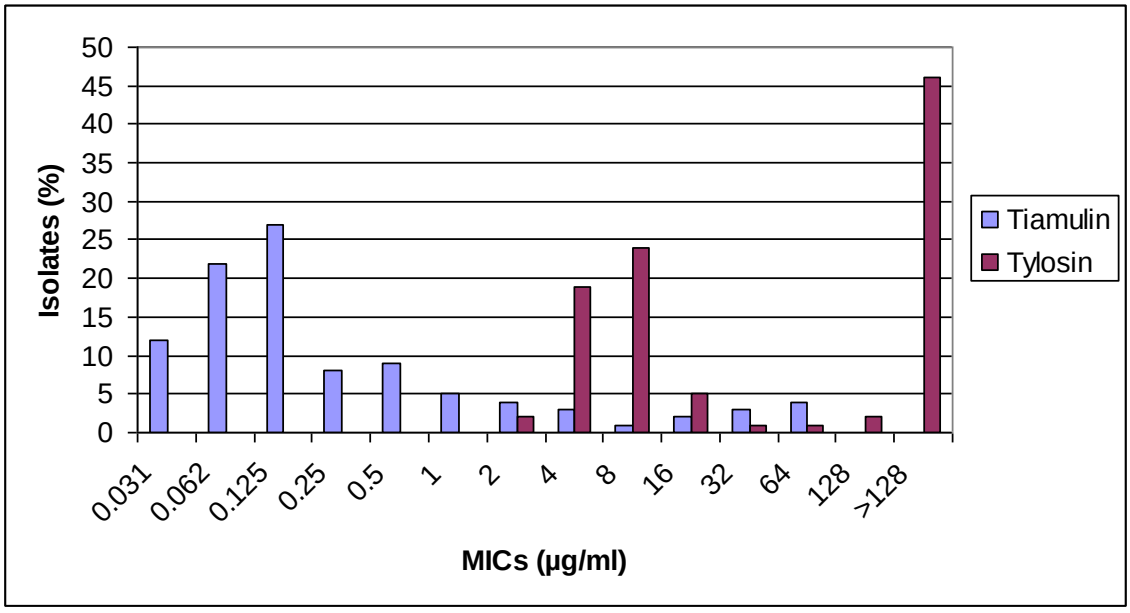
Table 3.20. *In-vitro* activity of various antibiotics against 25 US field isolates of *B. pilosicoli* (*Kinyon *et al*, 2002)

Antibiotic	MIC 50% (µg/ml)	MIC 90% (µg/ml)	Range (µg/ml)
Valnemulin	0.06	0.5	0.03 – 2.0
Tiamulin	0.125	1.0	0.6-8.0
Lincomycin	32	64	4.0 - >128
Tylosin	>512	>512	<16 - >512

There are minor differences for the valnemulin and tiamulin levels - some isolates do exceed lincomycin's maximum CCC in the range figures and tylosin could be classed as resistant, even at the MIC 50 level.

Pringle *et al* (2006) also demonstrated a similar trimodal susceptibility pattern for tiamulin against 93 isolates of *B. pilosicoli* from Sweden and the typical bimodal results for tylosin.

Figure 3.35. Susceptibility patterns for tiamulin and tylosin against *B. pilosicoli* (Pringle *et al*, 2006)

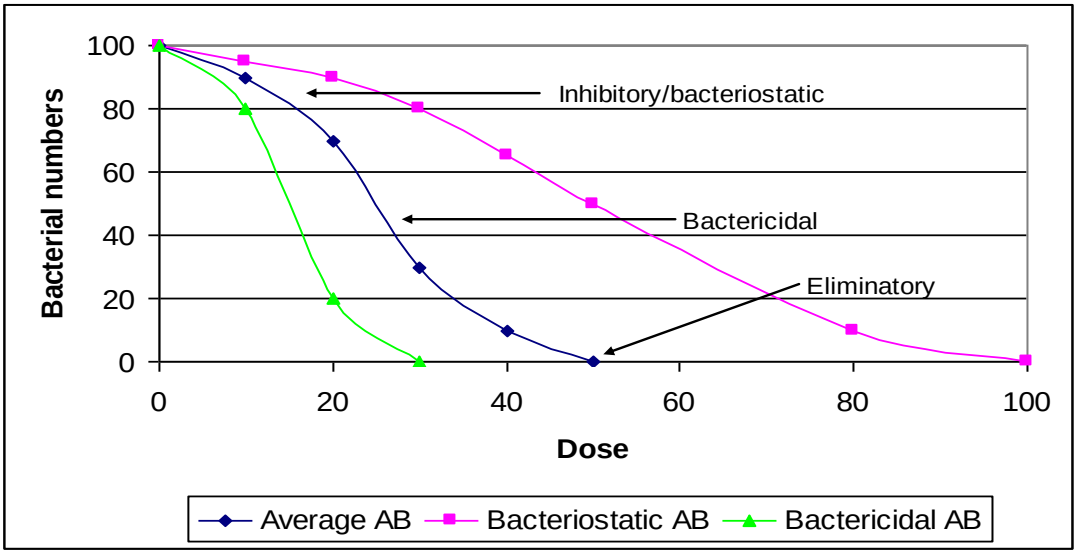


Clinical correlation of dose, effect and MIC/MBC

Dose-titration challenge studies using a known MIC/MBC of organism are the most useful for correlation purposes. For swine dysentery (*B. hyodysenteriae*) and colonic spirochaetosis 'colitis' (*B. pilosicoli*), there are two main types of study - prevention and treatment. In prevention studies, the antibiotic is administered before, at, or soon after infection, before clinical signs have appeared and some colonisation may have occurred, whereas treatment occurs when clinical signs or shedding of the organism is occurring, usually 7-21 days after infection. Normally with treatment trials, there is the treatment period of 7-21 days, followed by an observation period of 2-3 weeks, to see if the disease has been eliminated and the organism completely destroyed, or whether recrudescence occurs. This is usually confirmed by autopsy and culture of the colon. In prevention studies, there are sometimes no follow-up observation periods and autopsy may be performed at the end of the trial. This may mean that the organism is not completely eliminated, but inhibited to such a level not to cause gross visible lesions, but may still be present below the limit of detection or recovery by culture.

A classic dose/effect curve demonstrates the different responses seen usually between bacteriostatic and bactericidal antimicrobial drugs. As the dose increases, initially there is an inhibitory or bacteriostatic effect, then a bactericidal effect and finally, an eliminatory effect. If an antibiotic is more bactericidal in action, the curve is steeper, if it is more bacteriostatic in action, the curve is flatter. Most bacteriostatic antibiotics do show bactericidal tendencies at very high concentrations but in the case of tiamulin, valnemulin, lincomycin and tylosin the MBC/MIC ratios are very low, approximately two (Buller and Hampson, 1994; Rohde *et al*, 2004), hence they may be used to eliminate infections caused by *Brachyspira* species.

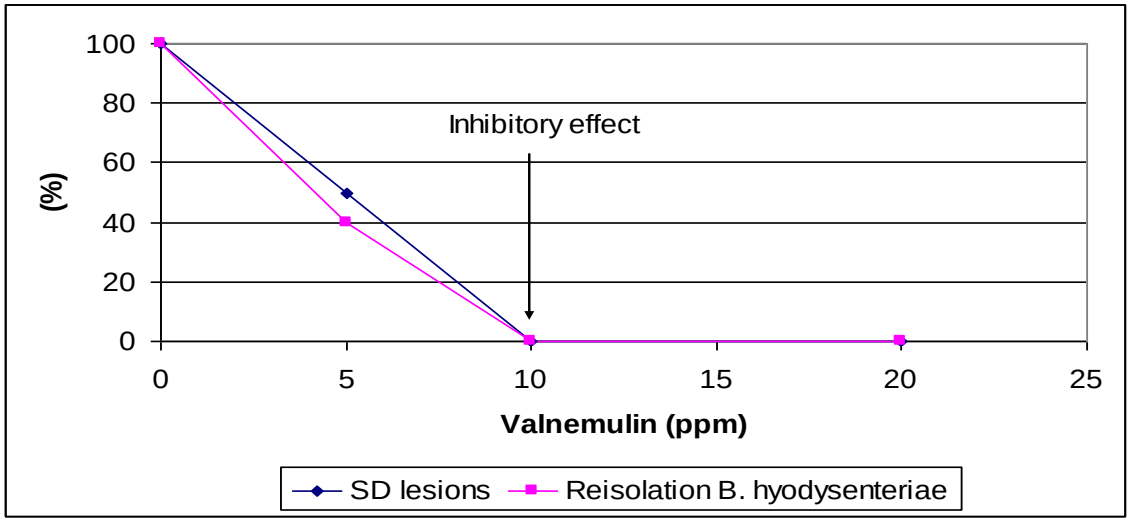
Figure 3.36. Classic dose/effect curves for antimicrobial substances (*Burch 2005a)



Valnemulin

*Burrows *et al* (1996a) showed, in an artificial challenge study using an isolate of *B. hyodysenteriae* with an MIC of 0.025µg/ml using an agar plate method that10ppm valnemulin inhibited the growth of the isolate of *B. hyodysenteriae* and prevented lesions and clinical disease. The medication started the day following challenge and lasted for 21 days; there was no observation period, so one could not say absolutely that it had eliminated the organism. In fact, in the same paper, pigs on higher levels of valnemulin broke down with dysentery when afflicted with a concurrent disease problem and they had stopped eating. One can only assume that it was having a bacteriostatic effect and not a bactericidal/eliminatory effect.

Figure 3.37. Inclusion rate/effect study with valnemulin in feed for the prevention of swine dysentery (*Burrows *et al,* 1996a)



In a treatment trial (*Burrows *et al*, 1996b), the pigs were treated for 10 days after disease developed in the group and there was a 14-day observation period. Although there was a good clinical response (bactericidal effect) at 75ppm valnemulin (the recommended use level), the organism was only eliminated from all of the pigs at 100ppm and above.

Figure 3.38. Inclusion rate/effect study with valnemulin in feed for the treatment of swine dysentery (*Burrows *et al*, 1996b)

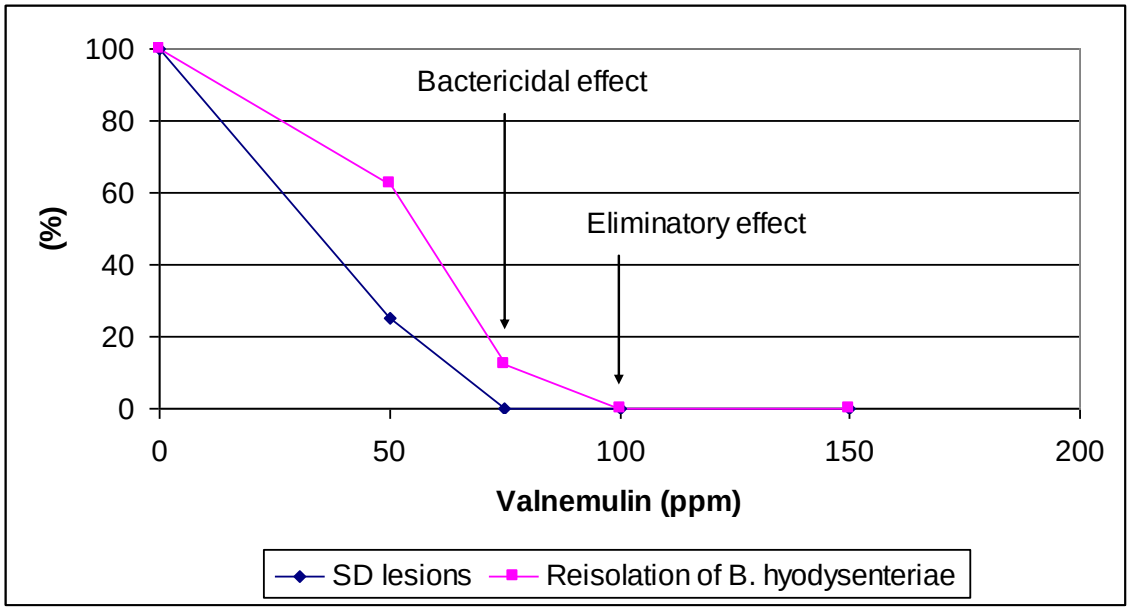


Table 3.21. Correlation of clinical effect of valnemulin with CCC, AUC and MIC/MBC determined by broth and agar methods (MIC 0.025µg/ml) (Calculations DB)

Valnemulin (ppm)	Colonic contents concentration (CCC) (µg/ml)	AUC (µg.h/ml)	AUC/MIC (h)	AUC/MIC less PPB of 87.5%
10 (prevention)	0.24	5.76	230	29
75 (treatment)	1.68	40.3	1612	202
100 (treatment)	2.4	57.6	2304	288

Key: PPB = plasma protein binding 87.5%

From these calculations, there is a very high AUC/MIC figure at 10ppm for just a bacteriostatic effect. Normally one would expect a figure approaching 24. Similarly, this is the case for the bactericidal and eliminatory effect. Valnemulin is strongly **plasma-protein bound (PPB)** to the order of **87.5%,** allowing 12.5% of active drug to be available. This suggests that there may be a high level of faecal/protein binding in the colonic contents. If the AUC/MIC figures are reduced by 87.5% then the AUC/MIC ratio is 29, 202 and 288 for 10, 75 and 100ppm valnemulin respectively, which would be closer to normally acceptable levels for bactericidal drugs. Interestingly, the MIC was derived from an agar plate test and therefore is likely to be the MBC.

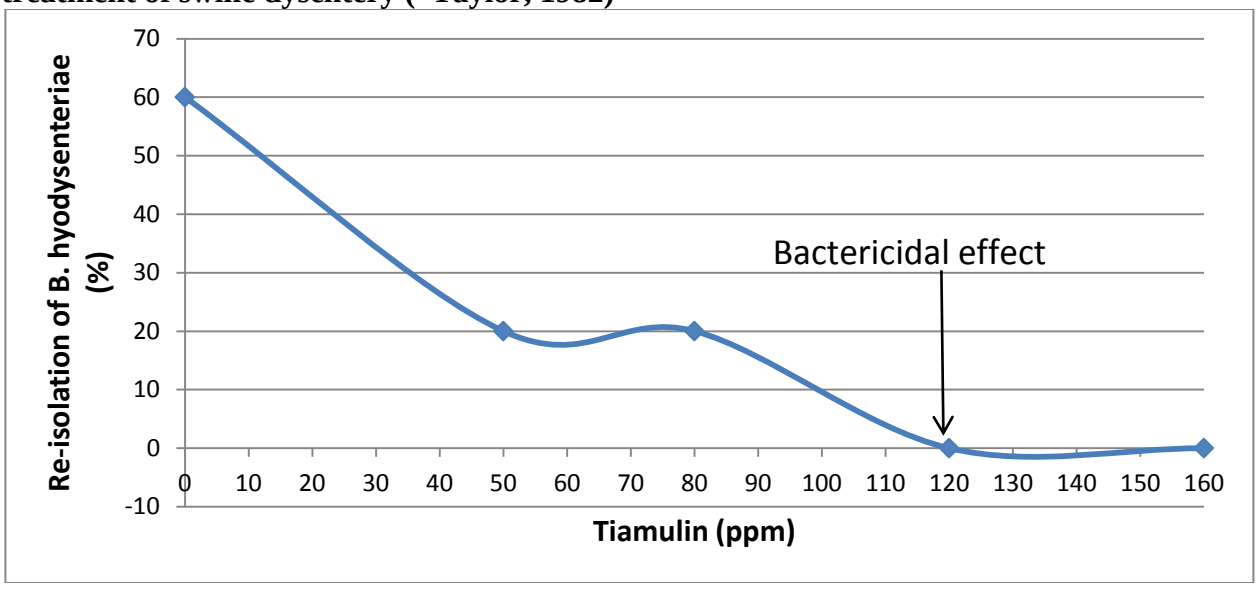
This suggests that if effective bio-concentrations can be achieved of 0.025µg/ml in colonic contents at 10ppm valnemulin then at 75-100ppm they will be about 0.188 to 0.25µg/ml. These concentrations coincide with the first dip in the susceptibility pattern with an ECOV of 0.125-0.25µg/ml (Karlsson *et al*, 2002). The product can be used up to 200ppm, which would give an **effective inhibitory bio-concentration** or **clinical breakpoint** of **0.5µg/ml**.

Tiamulin

Taylor (1980) described a dose-titration challenge study for the prevention of swine dysentery. The MIC of the isolate of *B. hyodysenteriae* (isolate S73/2) used was determined by the broth method at 0.05µg/ml (*Taylor, 1976). The pigs were given tiamulin medicated feed (0, 25, 30, 35 and 40ppm) from 5 days before infection for 42 days post-infection. The pigs were infected on day 0 and 1. Autopsies were carried out following a 21-day observation period on unmedicated food. No dysentery occurred during the trial and *B. hyodysenteriae* was not recovered from the faeces during or after treatment. However, on autopsy, it was recovered from colon scrapings in 2/5 pigs in groups given 35 and 40ppm tiamulin, suggesting it was not an eliminatory level but a bacteriostatic level.

In a treatment study, pigs were infected with a different isolate of *B. hyodysenteriae* (isolate S80/5) with an MIC of 0.5µg/ml by a broth method (*Taylor, 1982). The pigs were challenged three times and treated when clinical signs of dysentery developed. Tiamulin was given at 0, 50, 80, 120 and 160ppm for 14 days in the feed and the pigs autopsied 21 days after the end of treatment. Pigs in treatment groups 120 and 160 ppm responded quickly to treatment and *B. hyodysenteriae* were not isolated in the treatment or observation period, but were from the majority of pigs at autopsy.

Figure 3.39. Inclusion rate/effect study with tiamulin in feed for 14 days for the treatment of swine dysentery (*Taylor, 1982)



These levels showed a clear bactericidal activity but not an eliminatory effect. In contrast, the 50 and 80ppm groups showed some clinical signs throughout the trial and also *B. hyodysenteriae* could be isolated. At autopsy 3 weeks after treatment, *B. hyodysenteriae* could be isolated from almost all of the pigs. An inhibitory and bactericidal effect was seen at these inclusion rates. The **plasma-protein binding** for tiamulin is approximately **30-40%** depending on concentration.

Table 3.22. Correlation of clinical effect of tiamulin with CCC, AUC and MIC determined by a broth method (Calculations DB)

Tiamulin (ppm)	Colonic contents concentration (CCC) (µg/ml)	AUC (µg.h/ml)	AUC/MIC (h)	AUC/MIC less PPB of 40%
MIC 0.05µg/ml (S73/2)				
25 (prevention)	0.78	18.7	749	449
MIC 0.5µg/ml (S80/5)				
80 (treatment)	2.49	60	120	72
120 (treatment)	3.74	90	180	108
160 (treatment)	4.98	120	240	144

Key: PPB = plasma protein binding 40%

The AUC/MIC is very high in the prevention trial with 25ppm tiamulin at 749h. The inclusion rate had not been titrated lower and the isolate used was very sensitive, consequently the response was very good. In the treatment study, the AUC/MIC figures at 120 and above were close to expected levels for a bactericidal effect of 180h. If a figure for plasma-protein binding is used to represent potential faecal binding the level comes down to 108h, which is in between the 100-125h standard used for bactericidal drugs. However, if an MBC was used of twice the MIC (broth) (Buller and Hampson, 1994) the figures fall to 54 and 72h, which are only just becoming bactericidal.

Tiamulin at 40ppm gives approximately 1.0µg/ml of tiamulin in colonic contents. The wild type breakpoint is at 0.5µg/ml and using the plasma-protein binding figure of 40%, a bio-effective concentration for prevention would appear to have been reached at this point. Higher concentrations in feed would be necessary to control the first stage mutants.

Lincomycin

*Cowan and Duhamel (1997) described a dose-titration study for the treatment of colonic spirochaetosis caused by an artificial challenge with *B. pilosicoli* with a reported MIC of 50µg/ml using an agar plate method (J. Kinyon, personal communication). Two weeks post-infection, the pigs were treated with feed containing 0, 22, 33 and 110ppm of lincomycin for three weeks and there was a two-week observation period. Autopsies on the pigs were not performed. There were clinical improvements in the 33 and 110ppm groups and *B. pilosicoli* shedding stopped in these groups after the first week of

treatment and for the rest of the study. In the 22ppm group, diarrhoea continued throughout the study and *B. pilosicoli* was isolated each week and had no disease control effect.

Figure 3.40. Inclusion rate/effect study with lincomycin in feed for the treatment of colitis (*B. pilosicoli*), 7 days after the start of treatment (*Cowan and Duhamel, 1997)

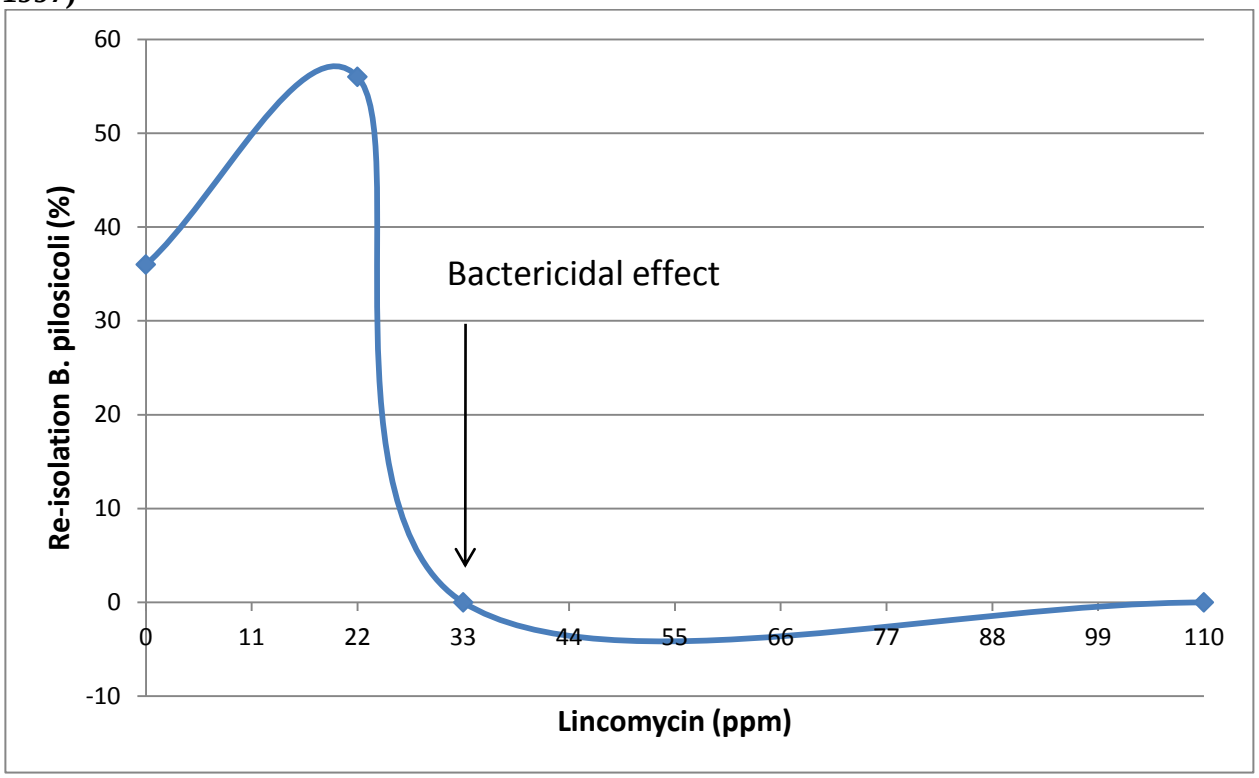


Table 3.23. Correlation of clinical effect of lincomycin with CCC and MIC determined by an agar-plate method (MIC 50µg/ml) (Calculations DB)

Lincomycin (ppm)	**CCC (µg/g)**	AUC (µg.h/ml)	AUC/MIC (h)
22 (no effect)	8.5	204	4.1
33 (bactericidal)	12.7	305	6.1
110 (bactericidal)	38.2	917	18.3

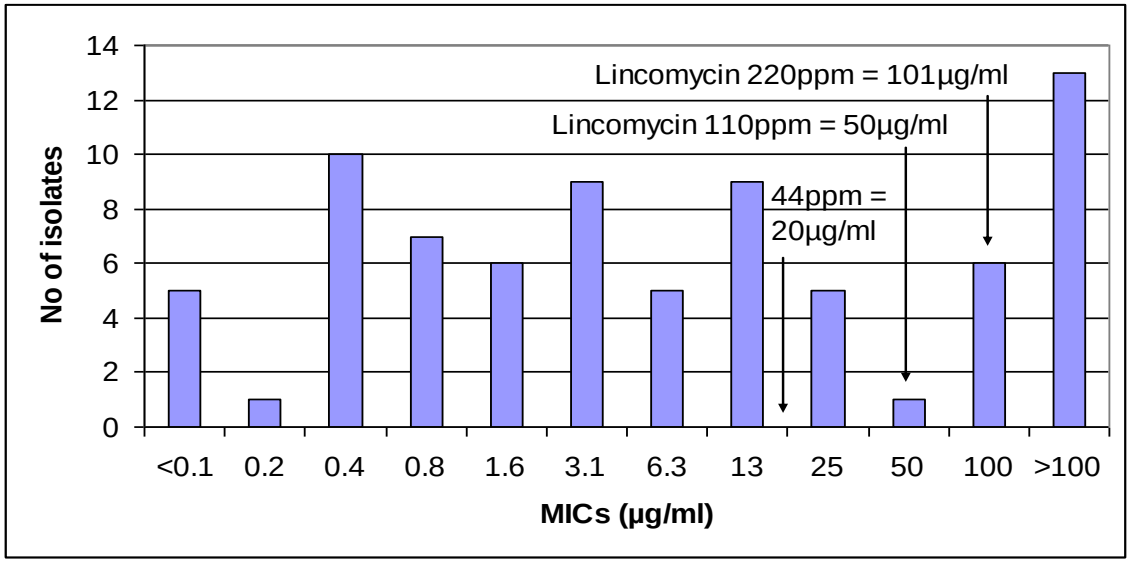
These results are in complete contrast with earlier findings with the pleuromutilins, where much higher CCCs were found in comparison with the MICs. Even using the MIC at 25µg/ml, the CCC does not become exceeded until 110ppm, and it is difficult to explain how the 33ppm worked so well. It would suggest that either the MIC figure is high, or the colon concentrations are too low, or another action is taking place. The MIC was determined by agar plate test, which is commonly higher than the broth tests by one dilution (Rohde *et al*, 2004) and thought to be the MBC, so the MIC could be between >12.5-25µg/ml. The *DeGeeter *et al* (1980) paper also has a possible discrepancy between the comparative ileal and colon contents concentrations between the two dose rates 110 (38.2µg/g) and 220ppm (101µg/g) and may have lowered the average CCC

figure, which should be approximately 15µg/g. Once the adjusted MIC and pharmacokinetic factors are tailored into the equation, the equivalent AUC/MIC ratio increases to 29h at 33ppm lincomycin, which should be a bacteriostatic level. This suggests that the effective inhibitory concentration at 110ppm lincomycin could reach 50µg/ml and at the maximum recommended usage level, 220ppm, 100µg/ml by micro-broth test. This could account for the shifting resistance trend pattern seen. *Brachyspira pilosicoli* may be more easily controlled as the inflammatory reaction is less severe and less protein-rich inflammatory debris (including blood) is produced.

This suggests that the effective inhibitory bio-concentration in the colon is closer to the actual CCC (CCC/MIC ratio of 1) in contrast to the pleuromutilins. One additional factor may be the comparatively low plasma-protein binding of lincomycin at 4% reported in Anon. (1998) unlike tiamulin at 30 and 40% and valnemulin at 87.5%. This is a particularly low figure, as other species have a plasma protein binding of 30-40% (Anon., 2003a) but would be supported by the clinical data. With reference to lincomycin's susceptibility pattern, it would suggest that the first wave is of highly sensitive organisms and the second wave may still be all susceptible to an inhibitory effect, depending on the level of lincomycin included in the feed. There would appear to be a number of high MIC isolates of *B. pilosicoli*, >128µg/ml (*Kinyon *et al*, 2002), which might suggest actual resistance. This was confirmed by Vyt and Hommez (2006) that 110ppm lincomycin did not control isolates with MICs of 128µg/ml and above but did up to 64µg/ml.

This was further confirmed by work reported by *Adachi *et al* (2008) looking at 91 Japanese isolates of *B. hyodysenteriae* and determined their MICs with the agar plate method. The major breakpoint was at 50µg/ml before resistance mutation developed.

Figure 3.41. Susceptibility pattern of lincomycin against 91 Japanese isolates of *B. hyodysenteriae* (CCCs based on 220ppm data) (*Adachi *et al,* 2008)



Tylosin

There is no recent dose-titration study data available for tylosin to make any determinations. It is well recognised that the majority of isolates of *B. hyodysenteriae* and *B. pilosicoli* are resistant to tylosin. From Karlsson et al (2002), there is a clear bi-modal wave presented. This suggests that the isolates with an MIC of up to 16µg/ml are the wild type and are susceptible, but there has been a mutational jump from there to the 256µg/ml resistant isolates. It can be seen that the bio-effective concentrations achieved in the colon are in the order of 14µg/ml when administered at 40ppm tylosin and 34µg/ml at 100ppm in the feed and this has driven the single resistance mutation step to ≥256µg/ml.

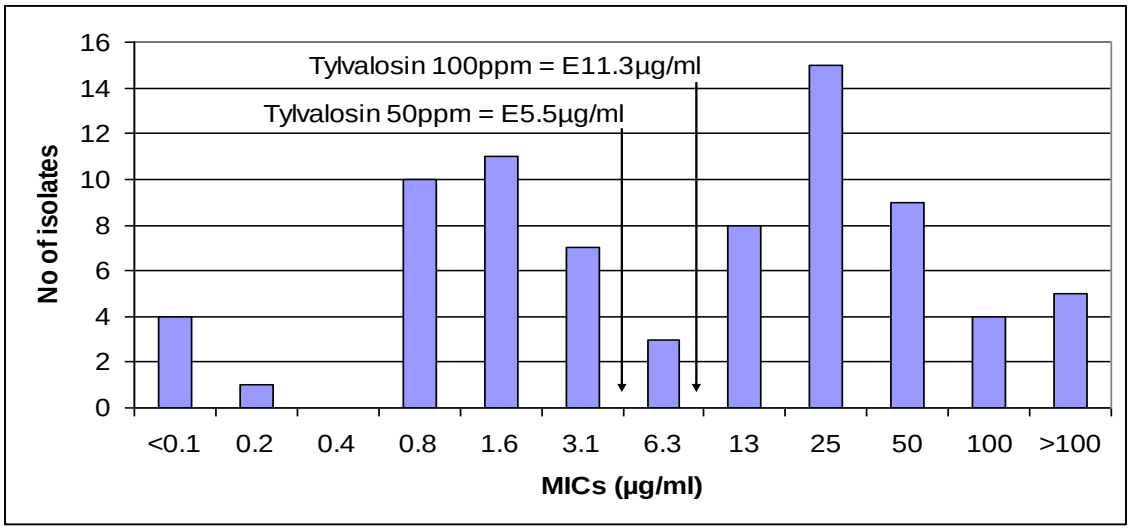
A plasma-protein figure is not available for pigs but is of the order of 30-40% in other species (Anon., 2003b).

Tylvalosin

Tylvalosin has recently been reported for the prevention (50ppm) and treatment (100ppm) of swine dysentery (*Tasker *et al*, 2004) but unfortunately there are no dose-titration or challenge strain MIC results publicly available. *Cizek *et al* (2003) reported that clinical efficacy was seen against isolates with MICs of 12.5-25µg/ml by agar plate test.

More recent data from Japan (*Adachi *et al*, 2008) where tylvalosin has been used for nearly 20 years, demonstrated a different susceptibility pattern using agar dilution against 91 *B. hyodysenteriae* isolates.

Figure 3.42. Susceptibility pattern of tylvalosin against 91 Japanese isolates of *B. hyodysenteriae* (*Adachi *et al*, 2008)



This correlates quite well with the average CCC estimated from the radio-labelled studies of 5.5µg/g for 50ppm and 11.3µg/g for 100ppm (upper CCC ranges 8.8-

17.5µg/g, respectively) giving a CCC/MIC ratio of about one. It does not include any activity associated with microbiologically active metabolites, so the effective CCC may be slightly higher. A resistance mutation appears to be taking place at >6.3µg/ml. The plasma protein binding is approximately 40% in pigs (J. Tasker – Personal communication) similar to tylosin.

Conclusions

The data cannot be considered complete and there are still many variations in studies, methods of MIC determinations (doubling dilutions, broth or agar culture), effect of faecal/protein binding, so absolute precision cannot be completely achieved. However, a pattern appears to be developing, which may help the clinician determine the clinical breakpoint for a particular isolate, against a particular antibiotic by a particular MIC method and select an effective dose rate.

The estimated **clinical inhibitory breakpoints** are summarised below. They do not reflect bactericidal or eliminatory levels that might be achieved with the in-feed inclusion levels represented.

Table 3.24. Clinical inhibitory breakpoints (MICs) for *Brachyspira* spp by both micro-broth and agar plate methods (Agar plate tests approximately double broth and micro-broth tests) (Calculations and analysis DB)

Antibiotic (level in feed ppm)	Micro-broth test MIC breakpoint (µg/ml)	Agar plate test MIC breakpoint (µg/ml)
Valnemulin (75)	>0.2	>0.4
Tiamulin (100 UK) Tiamulin (200 AUS, 220 US)	>1.6 >3.2	>3.2 >6.4
Lincomycin (110)	>25	>50
Tylosin (100)	>20	>40
Tylvalosin (100)	>8	>16

*Rønne and Szancer (1990) previously reported their estimations for breakpoints using agar plate tests only.

Table 3.25 Estimations for MIC breakpoints for *B. hyodysenteriae* using agar plate tests in Denmark (*Rønne and Szancer, 1990)

	MIC breakpoints (µg/ml)		
Antibiotic	**Sensitive**	**Intermediate**	**Resistant**
Tiamulin	≤1.0	>1.0 to ≤4.0	>4.0
Lincomycin	≤4.0	>4.0 to ≤36	>36
Tylosin	≤1.0	>1 to ≤4	>4

The breakpoints for tiamulin are relatively similar to the *Rønne and Szancer (1990) results. With regard to lincomycin, the breakpoint is approximately 2 times higher in this study, but their breakpoint was based on the *DeGeeter *et al* (1980) data as well, which may have an underestimation of CCC at the 110ppm level. The tylosin data by contrast

relates to colon tissue, rather than colon contents, following injection and would appear to be much lower than the present study, which takes into account CCC and MIC patterns, although there is a dearth of good clinical data to support it.

As more data becomes available and standardisation is introduced, clearer interpretations may be made. It is a difficult area to work in, as there are so many variables to take into account. However, as the cultural methods become more standardised and more information on gut pharmacokinetics becomes available, which can then be correlated with clinical work, more precise determinations can be made.

Chapter 4. Overall Conclusions and Discussion

Pig meat production is an important source of protein around the world. To meet demand intensification has increased over the years and as a result disease pressures have often increased and spread around the world, such as PRRS virus and PCV2, in spite of husbandry and management improvements, vaccine development and antimicrobial use. Antimicrobial use in pigs is on a par, if not higher than poultry or cattle production. In-feed medication is also one of the most important routes of administration of antibiotics in pigs because of its ease of use and most of the bacterial infections become predictive and occur at certain stages of production, so they are routinely used.

The understanding of the use of these products by veterinarians is sometimes limited but generally they work well according to the directions given in their Summary of Product Characteristics, although some of the data are quite old. Improvements in veterinary knowledge may help better drug selection, reduced and more targeted usage, correct dose administration and, as a result, improved treatment results and less resistance development.

Understanding of the pharmacodynamics of the antibiotics helps selection of the correct antibiotic to treat the right disease and knowledge of the pharmacokinetics of the drug helps target it to the right body system at an effective dose rate. The integration of both PD and PK really can help improve the understanding of how drugs work and improve clinical response and opportunities for bacterial elimination or eradication in some cases.

Regarding respiratory infections, PK/PD integration against *M. hyopneumoniae* demonstrated that plasma concentrations are the main PK indicator for efficacy correlation. Although *M. hyopneumoniae* culture and MIC testing has not been standardised by the CLSI, there is generally a consistency in the results achieved with similar methods. Responses to the bactericidal fluoroquinolones are consistent and predictable with regard to PK/PD integration. The tetracyclines are effective bacteriostatic antibiotics for prevention with high MBC/MIC ratios but no antibiotics appear to be completely eliminatory when lesions are established. The majority of antibiotics like the macrolides, pleuromutilins and lincosamides are mainly inhibitory in their action. The macrolides and pleuromutilins also concentrate in lung tissue to a high degree and this may impact local plasma levels and extracellular fluid levels where the mycoplasmas mainly exist.

The fluoroquinolones and tetracyclines appear to act as anticipated against bacterial respiratory infections such as those caused by *A. pleuropneumoniae,* and PK/PD integration follows predictable results. There is a major discrepancy between PK and PD of macrolides and pleuromutilins against *A. pleuropneumoniae* and the reasons are not completely clear. From a PD point of view there are great variations in the MIC results depending on method of culture. There is a standardised CLSI method but the results from this bear no relation to plasma concentrations of the macrolides and pleuromutilins. Whether it is the PD method i.e. the presence of serum or the pH of the medium, which

can also alter the pKa balance of the antibiotic, is not clear. There are suggestions that there might be an alternative mode of action with these antibiotics. It is thought that lung concentrations are unlikely to be significant other than as a surrogate marker but the debate over the concentration of drug in the macrophage and other leucocytes such as neutrophils still seems the most likely mode of action against *A. pleuropneumoniae*. The antibiotics, particularly tilmicosin, tulathromycin and tiamulin have been shown to concentrate at high levels in these cells and it is felt that this may be the most likely explanation for efficacy.

Regarding enteric infections, the enteric model described in Chapter 3 seems to be applicable to not only non-absorbed antimicrobial agents but also to ones that are absorbed. The PK calculations based on colon contents or faecal concentration give a good correlation with antimicrobial susceptibility patterns developed for *E. coli* and *L. intracellularis*. The absolute PK/PD relationship, suitable for precise modelling of a drug dose are not refined enough yet but the relationship of between approximately 25-30% of the colon contents concentration in the small intestine for absorbed and non-absorbed compounds, developed by the author (DB), appears to fit well.

The original PD or intracellular MIC for *L. intracellularis* (McOrist *et al*, 1995) appear to be limited due to the few strains used and the narrow range of MICs tested. This made PK/PD integration and interpretation originally quite difficult. With the new data from Wattanaphansak *et al* (2009) it appears to have overcome the initial problems and integration is explained and even susceptibility patterns confirm the in-vivo findings. There were differences between the artificial infection challenge models and the severity of disease that could be caused. The cell culture model (McOrist) was milder than the ground-up ileum challenge model (Winkelman), which caused on occasions a high level of mortality and extensive lesion development beyond what is commonly encountered in the field. It was felt that the McOrist model was more suitable for dose-determination studies and the author (DB) felt better related to the challenge in the field.

Regarding *Brachyspira* spp infections in the colon, there was a broad approximation of PK/PD integration. The determination of *B. hyodysenteriae* MICs via broth or agar plate also had an effect on the final MIC achieved. The methods have not been standardised by CLSI, so again this can lead to some variation between authors. The author (DB) found that there is an improved correlation between clinical efficacy and the agar plate-derived MICs because they appear to be equivalent to the MBCs. It was also interesting to find that the MICs for primarily bacteriostatic drugs were often only one dilution different from the MBCs. Integration of PK/PD with clinical trial results showed that there were some major discrepancies in inclusion rate and efficacy. It appeared that faecal or protein binding may have played an important role. Valnemulin has a high plasma-protein binding (87.5%) and the bio-effective concentrations achieved in the colon were relatively low in comparison with a related compound tiamulin, which has a moderate plasma-protein binding of 30-40%. The bio-efficacy of lincomycin against *B. pilosicoli* indicated that the binding of the drug was low, as it was difficult to correlate PK with PD and clinical effect. The plasma-protein binding of lincomycin had been reported to be as low as 4% in the pig. When binding was taken into account the PK/PD relationship figures became more in line with the standards. The author (DB) found that

susceptibility patterns of MICs also supported the PK/PD relationships, but it was apparent that resistance to the few remaining antibiotics effective against *B. hyodysenteriae* was developing in some countries.

Overall, there is still some way to go to solve some of the relevant PK and PD aspects in pig respiratory and enteric infections and to improve their accuracy. Standardisation of MIC/MBC determination methods would be of help as well as cross-over MIC determinations to reduce the potentially large variation caused by doubling-dilution techniques. Improved pharmacokinetic information using chemical analysis of active substance and active metabolites, at the site of infection, would also be of great help. Clinical dose-titration studies with reported MIC challenge organisms are also essential for final interpretation and integration of the results.

It is hoped, from this extensive review of the literature and the in-depth PK/PD analysis of the available data to confirm the role that such analysis can have and identify the current deficiencies in the information available, that this thesis will form the basis for further work into improving our knowledge and understanding of how antibiotics work in pigs and how the veterinarian can best employ them to improve clinical results.

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