ANTIMICROBIAL CONCENTRATIONS IN PLASMA AND LUNG AND THEIR RELATIONSHIPS TO BACTERIAL RESPIRATORY INFECTIONS

D.G.S. BURCH Octagon Services Ltd, Old Windsor, Berkshire, UK.

The Pig Journal (2010) 63 34-49

Summary

Some antimicrobials, such as tiamulin, tilmicosin and tulathromycin, have been shown to concentrate in lung tissue and have also been reported to have shown good efficacy in the control of respiratory bacterial infections in the pig. The most common infection is caused by Actinobacillus pleuropneumoniae (App). Classical pharmacokinetic and pharmacodynamic relationships using plasma concentrations do not appear to apply, as the plasma concentrations for these substances are usually much lower than the minimum inhibitory concentration (MIC). It was the purpose of this paper to explore the relationship of lung concentrations in comparison with the MICs of the major respiratory bacteria, such as App as well as Pasteurella multocida (Pm) and Haemophilus parasuis (Hps) and correlate these with the results of artificial challenge studies and antimicrobial susceptibility/resistance patterns from field isolates. Actinobacillus pleuropneumoniae is a fastidious organism and its MICs can vary substantially with the culture method employed. Tiamulin administered in the drinking water shows good efficacy against App and the lung concentration correlated well with the MIC of the organism used and an epidemiological cut off value (ECOV) occurred around 8.0µg/ml and could be observed for App and Hps but not for Pm. Tilmicosin also showed an ECOV at 8.0µg/ml, which correlated with alveolar macrophage concentrations rather than lung concentration for Pm and Hps but not for App. This was thought to be due to the MIC determination and culture method, as strains with MICs of 16µg/ml were treated successfully. Tulathromycin's ECOV was approximately 4.0µg/ml for Pm and Hps and correlated with lung concentration but not for App. Again there was great difficulty ascertaining the susceptibility of App, as there was a large variation in MIC depending on the method and conditions used. The MIC was markedly reduced when serum was included in the culture media. Plasma levels correlated well with Mycoplasma hyopneumoniae MICs and serum is also included in the medium as a routine. Recent data suggests that tiamulin's MICs against App are also substantially reduced when serum is added to the medium. Further work is required to clarify these MIC anomalies.

Introduction

The pharmacokinetic (PK) and pharmacodynamic (PD) relationships for bactericidal antimicrobial compounds to achieve good bacterial kill and clinical response have been determined. For aminoglycosides and fluoroquinolones a ratio of concentration maximum (Cmax) in plasma divided by the minimum inhibitory concentration (MIC) for the organism should be approximately 10-12 (Toutain, 2003). An alternative relationship, using the area under the curve (AUC) achieved by the antimicrobial in plasma over time (usually 0-24 hours) divided by the MIC of the organism gives a ratio of 100-120. This also applies to the aminoglycosides, fluoroquinolones and importantly the penicillins and cephalosporins, which are both concentration and time dependent in their bacterial killing effect.

These basic PK/PD relationships work well when the MIC is similar to the minimum bactericidal concentration (MBC) but when the MBC/MIC ratio is much higher, *e.g.* for bacteriostatic drugs such as tetracyclines, macrolides, lincosamides and pleuromutilins then the classic PK/PD relationships can be markedly distorted. However, they can be restored by the use of the MBC in the calculations. This was shown to be the case for various antimicrobials against *Mycoplasma hyopneumoniae* (Burch, 2004).

Some antimicrobials accumulate in high concentrations in lung tissue in comparison with their plasma levels, *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). It is the purpose of this paper to explore the relationship of antimicrobial lung concentrations and their MICs against the common porcine respiratory bacteria, such as *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* in comparison with *M. hyopneumoniae* and their effect on clinical efficacy and antimicrobial susceptibility patterns.

Plasma and lung pharmacokinetics of various antimicrobials

Plasma levels are still considered the primary pharmacokinetic parameter, but related to that is the concentration of an antimicrobial that can be achieved in the lung. The actual level in the lung depends on a number of factors such as lipid solubility and cell membrane penetration, as well as dissociation constants pKa and subsequent ionisation and entrapment in the alveolar cell. The majority of substances that concentrate in lung tissue are usually weak bases, which become more ionised in the slightly higher acid conditions intracellularly. However, plasma and lung levels are dynamic and flow both ways and possibly the lung may even act as a local reservoir, prolonging activity but may also act as a drain, limiting local plasma and extracellular fluid concentrations.

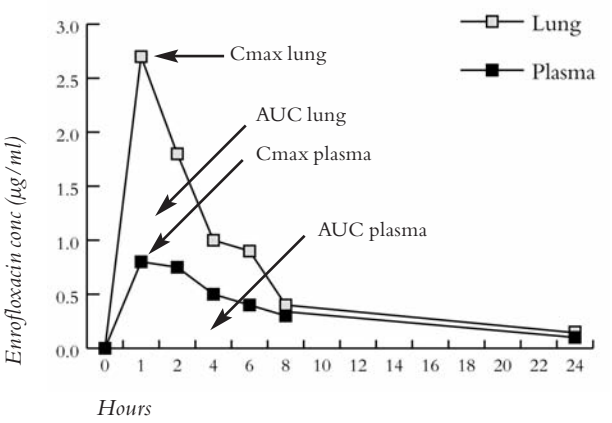
Enrofloxacin

Scheer (1987) reported on the concentrations of enrofloxacin in plasma and lung of pigs following an injection at 2.5 mg/kg bodyweight (see figure 1). A microbiological assay method was used for both plasma and lung tissues. This is important as it determines the antimicrobial activity rather than identifies a specific substance by usually high pressure liquid chromatography (HPLC). This may miss biologically active metabolites, ciprofloxacin in the case of enrofloxacin, which is also highly active. Enrofloxacin and the fluoroquinolones have become almost a benchmark for PK/PD analysis for bactericidal antimicrobials.

The Cmax lung for enrofloxacin was 2.7 µg/g the Cmax plasma was 0.8 µg/ml and the Cmax lung/plasma ratio was 3.4: 1. The AUC lung was 15.5 µg.h/g and the AUC plasma was 7.2 µg.h/ml but the AUC lung/plasma ratio was 2.2: 1. In some reports, there is only a single lung figure in relation to plasma. This is helpful but not always so accurate, as there is a possible lag effect in reaching a peak concentration in the lung from plasma, and there is also a lag effect often from lung back to plasma. The AUCs probably give a more accurate lung/plasma relationship.

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

Figure 1 – Plasma and lung concentrations of enrofloxacin following a single injection at 2.5 mg/kg bodyweight in pigs



Oxytetracycline

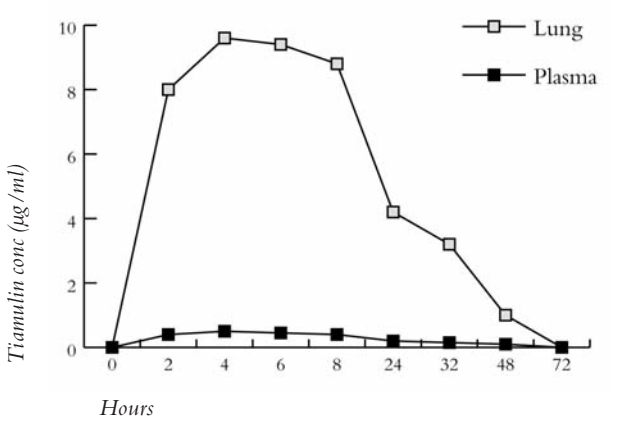
There are a number of references to oxytetracycline in plasma following in-feed administration but the most extensive work was by Pijpers *et al.*, (1990a and 1990b).

In the first study (Pijpers *et al.*, 1990a) they looked at levels of oxytetracycline in plasma following administration in the feed for six days at 400, 800, 1600 and 2400 ppm. The plasma samples were assayed using an HPLC and a microbiological method and relatively similar amounts were found. The highest concentrations of oxytetracycline were 0.22, 0.50, 1.43, and 2.14 µg/ml respectively. Recovery rates from spiked plasma ranged from 57-72%. In Pijpers *et al.*, (1990b), they looked at plasma and lung concentrations following administration of oxytetracycline in the feed at 400, 800 and 1600 ppm for six days. The pigs were also infected with *A. pleuropneumoniae* as part of an artificial challenge study. Slightly higher lung concentrations were found than in plasma (see Table 1) but slightly lower plasma levels were found than in the first study. The lung/plasma ratio varied between 1.09-1.36: 1.

Tiamulin

McKellar *et al.*, (2004), described the lung plasma relationships for tiamulin following an injection at 15 mg/kg bodyweight in pigs. The plasma and lung concentrations were assayed using a microbiological process again recording active substance and microbiologically active metabolites (see Table 2).

Figure 2 – Plasma and lung concentrations of tiamulin following a single injection at 15 mg/kg bodyweight in pigs



The Cmax lung for tiamulin was 9.6 µg/g the Cmax plasma was 0.61 µg/ml and the Cmax lung/plasma ratio was 15.7: 1. The AUC lung was 231.5 µg.h/g and the AUC plasma was 12.8 µg.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 medication at 38.5, 110 and 220 ppm and water medication at 60, 120 and 180ppm (see Table 1). Unfortunately, the comparative plasma levels were not reported.

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

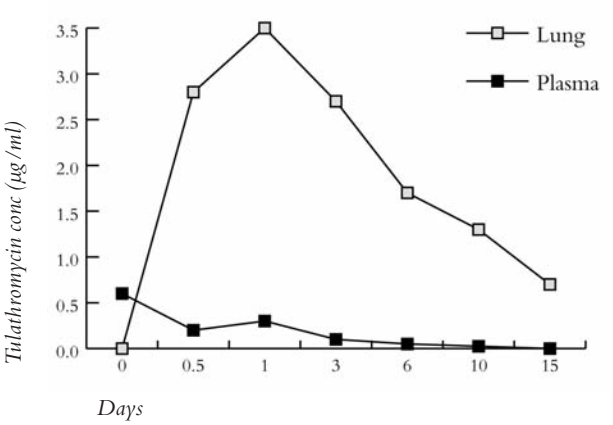
Tilmicosin

Thomson *et al.*, (1994) described the serum concentration of tilmicosin in relation to lung concentration after a period of feeding at 400ppm for 14 days. The serum and lung concentration peaked at about 10 days with levels of 0.23 and 2.59 µg/ml respectively by the HPLC assay method, which gave a lung/serum 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 20 µg/ml tilmicosin for 24 hours. Stoker *et al.*, (1996) showed that after feeding 400 ppm tilmicosin for 14 days average serum levels were 0.039, lung levels were 1.69, tracheal epithelium was 2.19 and lung macrophages were 7.19 µg/ml. The lung/serum ratio was 43: 1, which is substantially higher than the earlier work, 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 its lack of compliance with classical PK/PD assessments for the treatment of bacterial (*A. pleuropneumoniae*) respiratory infections that the real PK/PD debate over the significance of plasma levels and lung levels appeared to start in veterinary medicine.

Figure 3 – Plasma and lung concentrations of tulathromycin following a single injection at 2.5 mg/kg bodyweight in pigs (Benchaoui et al., 2004)



The Cmax lung for tulathromycin was 3.47 µg/g the Cmax plasma was 0.62 µg/ml and the Cmax lung/plasma ratio was 5.6: 1. The AUC lung (last time point 15 days) was 615 µg.h/g and the AUC plasma was 12.0 µg.h/ml but the AUC lung/plasma ratio was 51.3: 1, which is also substantially higher than enrofloxacin. At six days the AUC lung/plasma ratios were 29: 1. The assays were carried out by HPLC (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 (Evans, 2005).

Table 1 – Antimicrobial concentrations in lung and plasma and various ratios

Drug/Ref	Formulation	Dose (mg/kg)	Lung Cmax	Plasma Cmax	L/P ratio	Lung AUC	Plasma AUC	L/P ratio
Enrofloxacin								
Scheer, 1987 (M)	Inj (24 hr)	2.5	2.7	0.8	**3.4**	15.5	7.2	**2.2**
Premix data	In feed 150ppm	7.5	0.92	0.3	**3.1**	22.1	7.2	**3.1**
Ceftiofur								
Brown *et al*., 1999 (H)	Inj Na Cef Inj Cef HCl (72 hr)	3 3		15.8 11.8			196 216	
Tiamulin								
McKellar *et al*., 2004 (M)	Inj (72 hr)	15	9.6	0.61	**15.7**	231.5	12.8	**18.1**
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**			
Anderson *et al*., 1994 (M)	In feed 110ppm 220ppm	 6.6 13.2	 1.5 2.0	 0.08(E) 0.11(E)				
Nielson & Szancer, 1998					**PM/P 4.9 - 18.2**			
Tylosin								
Hoffman *et al*., 1983 (M)	Inj (24 hr)	10	3.37	3.49	**0.96**	21.3	16.1	**1.3**
Ibayashi *et al*., 1994 (M)	In feed 110ppm	5.5	<0.05	<0.03(E)	**Used 1.3**			
Tilmicosin								
Thomson *et al*., 1994 (H)	In feed 200ppm 400ppm	 10 20	 1.43 2.59	 <0.1 0.23	 **11.3**			
Stoker *et al*., 1996 (H)	In feed 400ppm	20	1.69 MPs 7.2	0.039	**43 MP/P 184**			
Blais & Chamberland, 1994					**MP/P 75**			
Tulathromycin								
Benchaoui *et al*., 2004 (H)	Inj LA form (15 days)	2.5	3.47	0.62	**5.6**	615	12.0	**51.3**
Evans 2005					**PM/P 16.6 MP/P 8.1**			
Lincomycin								
Swenson & Barbiers, 1976 (M)	Inj	11	12.5	7.03	**1.8**			
DeGeeter *et al*., 1980 (M?)	In feed 110ppm 220ppm	 5.5 11	 0.66 1.13	 0.16 0.14	 **4.1** **8.1**			
Oxytetracycline								
Banting & Baggot, 1996 (M)	Inj LA form (48 hr)	20		4.68			86.6	
Asanuma *et al*., 1986 (M)	In feed 400ppm	20	0.15	0.11	**1.4**	2.36	2.0	**1.2**
Pijpers *et al*., 1990a (H)	In feed 400ppm 800ppm 1600ppm	 20 40 80	 0.23 0.42 0.78	 0.25 0.57 0.83	 **1.09** **1.36** **1.06**			
Chlortetracycline								
Jacobson *et al*., 1994 (M)	In feed 1000ppm	50	0.56	0.44	**1.3**			
Asanuma *et al*., 1986 (M)	In feed 400ppm	20	0.66	0.35	**1.9**	11.75	5.78	**2**

Key – Inj = injection; M = microbiological assay; H = HPLC assay; E = Estimate; MPs = macrophages; Used = used in calculations

A number of antimicrobial products and their plasma and lung levels are highlighted in Table 1. Estimations (E) may also be included, where data is deficient.

In comparison with a bolus dose, such as an injection, when an antimicrobial is given in feed or drinking water over a 24 hour period, then the plasma levels and resulting lung levels are lower but flatter. The Cmax is usually lower, but the AUC dose for 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, especially where a microbiological assay method is used. Food usually slows the absorption but may or may not reduce the bioavailability or the AUC24hr unless the metabolism is mainly in the liver.

With bacteriostatic antimicrobials over time the antimicrobial concentration should be above the MIC and is the important measurement for efficacy. Therefore AUC divided by 24 (hours) gives the equivalent of a steady state effect for calculation purposes. For penicillins (concentration and time dependent) an AUC of 100-120 / 24 hours = 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 (one 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 the organism and the antimicrobial and can be several times higher.

Pharmacodynamics of various respiratory associated bacteria

Classically, the MIC of the antimicrobial against the organism is the important measurement of susceptibility. When we have a number of isolates (ideally 10 or more) then the MIC 50% for a population and MIC 90% and range can be determined and this is how they are normally expressed. This gives a broad indication of the susceptibility of the population, but it needs to be put in context of what are achievable antimicrobial levels, say in plasma or other target tissues and fluids.

A. pleuropneumoniae

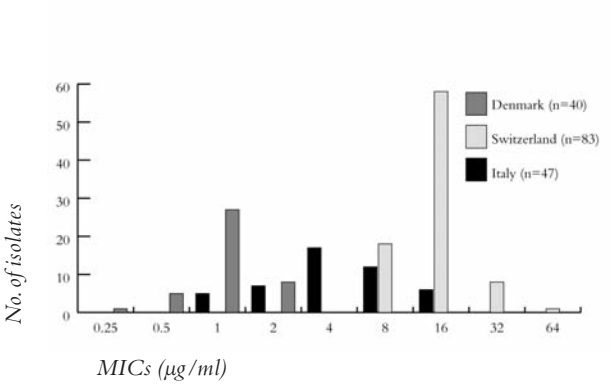
Table 2 shows the MICs of various antimicrobials against *A. pleuropneumoniae*.

Consistently, there is a high level of susceptibility to ceftiofur and in general enrofloxacin; although in Taiwan there is some degree of resistance development. The reported MICs for tiamulin are quite 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* (see Figure 4). There were no apparent resistance patterns developing. Recent work (Burch *et al.*, 2009) showed that the addition of 50% serum reduced median MICs of tiamulin against *A. pleuropneumoniae* by 6.6 times.

Table 2 – MICs of various antimicrobials against A. pleuropneumoniae

Antimicrobial / ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Aarestrup and Jansen, (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) – Tiawan 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

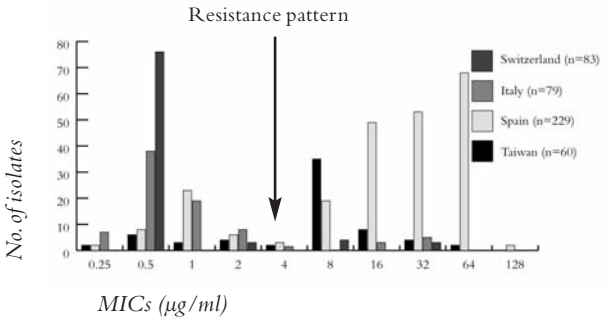
Figure 4 – Comparison of MIC results for tiamulin from different authors against A. pleuropneumoniae (Sidoli et al., 1984; Matter et al., 2007; Aarestrup and Jensen, 1999)



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

The susceptibility to tetracycline is also very variable with high levels reported in Taiwan (Chang *et al*., 2002) and Spain (Guttierrez –Martin *et al*., 2006), probably associated with resistance development in comparison with Switzerland (Matter *et al*., 2007) and Italy (Sidoli *et al*., 1984). The ECOV or 'wild type' MIC is consistently between 2-4 µg/ml and above is resistance.

Figure 5 – 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 its plasma and lung concentration and similarly lincomycin. Tilmicosin has reportedly high MICs, in line with its macrophage concentration and above but there is surprisingly 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

There have been relatively minor changes to the susceptibility of *P. multocida* over the last 14 years in Spain except to the tetracyclines, (See table 3). Spain does have a relatively high usage of antimicrobials in pigs but based on the injectable concentrations many isolates could be susceptible, but based on the in-feed PK levels achieved, there is potentially a high level of resistance (>10%). Tiamulin MIC 50s have reduced in the same period, but are still very high and probably out of therapeutic range for most isolates.

Table 3 – MICs of various antimicrobials 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

Haemophilus parasuis

Table 4 – MICs of various antimicrobials 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)			
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	8.0	≤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.

Mycoplasma hyopneumoniae

Table 5 – MICs of various antimicrobials against M. hyopneumoniae

Antimicrobial/ ref	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Inamoto *et al.*, (1994) – Japan 40 isolates			
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) – Belgium 21 isolates			
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 are some increases of MIC for some of the antimicrobials and resistance to tylosin, tilmicosin and lincomycin was reported in Belgium (<10%). Generally however, the MICs for tiamulin, tylosin, tilmicosin and lincomycin against *M. hyopneumoniae* are much lower than against the respiratory bacteria.

Clinical efficacy of various antimicrobials for bacterial respiratory infections

To make accurate assessments of PK/PD relationships to clinical effect, it is important to have the MICs of the organism used in the artificial challenge study. Unfortunately, these are not always published. Table 6 highlights the main indications of various antimicrobials.

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 150 ppm and then infected with the challenge strain four hours afterwards. Results are shown in table 7. The trial lasted for seven days when the pigs were necropsied. The lung lesion scores were reduced by 72% and 88% in the enrofloxacin 32 and 150 ppm treated groups in comparison with the controls. No *A. pleuropneumoniae* were isolated from the enrofloxacin 150ppm treated group, 17% of the 32 ppm group and 92% of the untreated controls. The MIC of the organism was given as >0.01 to <0.05µg/ml.

A better clinical response was achieved with the 150 ppm enrofloxacin and the control of the infection was very good, eliminating the challenge organism (see Table 7). The Cmax plasma/MIC was 10 and the AUC plasma/MIC was 240, which was approximately what would be expected for a fluoroquinolone. Surprisingly, the 32 ppm level was also quite effective in preventing lung lesions developing in the majority of pigs.

Oxytetracycline

The results are shown in table 8. Oxytetracycline was used in a number of studies (Pijpers *et al*, 1990b) which gave a dose titration effect. Pigs were given 0, 400, 800 and 1600 ppm prophylactically in feed and challenged with an isolate of *A. pleuropneumoniae* with an MIC of 1.0 µg/ml. The percentage of pigs with pneumonia was 100%, 67%, 27% and 0% respectively.

The MIC of the isolate could range from >0.5 to 1.0 µg/ml because of doubling dilutions, so the figure of AUC/MIC is approaching 24 (18.7 and 19.9) for both plasma and lung, suggesting that there is little difference between the two and the 1600 ppm of oxytetracycline is giving a good protection from the challenge infection. Additionally, the recovery of oxytetracycline was between 57-72%, so the final calculations may be underestimated.

Table 7 – Comparison of plasma and lung PK/PD relationships of enrofloxacin for the prevention of A. pleuropneumoniae

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

Table 6 – Main respiratory indications of various antimicrobials in the UK (NOAH, 2007)

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 / 10 days (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 21 days	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

Table 8 – Comparison of 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	AUC lung/ MIC
0	100	1.0	0	0	0	0
400	67		0.23	0.25	5.5	6.0
800	27		0.42	0.57	10.1	13.7
1600	0		0.78	0.83	**18.7**	**19.9**

Tiamulin

Tiamulin has also 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 0.1 μg/ml. Results are shown in table 9. Piglets were artificially reared on evaporated milk and infected at about one week of age. They were treated two weeks later with tiamulin at 10 mg/kg bodyweight given twice a day for 10 days and necropsied 14 days after treatment. Lung lesions in the control group were on average 24.5 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 water 180 ppm levels in the lung and plasma were used, as they were the nearest in dosage terms.

Table 9 – Comparison of plasma and lung PK/PD relationships of tiamulin for the treatment of M. hyopneumoniae

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

The Cmax and AUC plasma/MIC relationship calculations are approximately correct for good clinical efficacy, whereas the lung concentrations are largely in excess.

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 4.0 µg/ml. The results are shown in tables 10 and 11. Pigs were infected intranasally and when signs of disease started to occur they were allocated to the various tiamulin drinking water treatments at 0, 60, 120 and 180 ppm for five days. The pigs were necropsied 21 days after infection and their lung lesions scored and cultured for *A. pleuropneumoniae*.

Table 10 – Dose titration study with tiamulin administered in the drinking water for the treatment of A. pleuropneumoniae

Treatment Tiamulin (ppm)	MIC (µg/ml)	Mortality (24 hours)	Ave lung lesion score (%)	Ave lung lesions score of surviving pig	*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 11 – Comparison of plasma and lung PK/PD relationships of tiamulin for the treatment of A. pleuropneumoniae

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 would suggest that the lung Cmax and AUC / MIC relationships were the more important in comparison with the plasma for tiamulin and respiratory bacteria. Interestingly, the AUC lung/MIC of 25.8 correlated well with an inhibitory effect and that the AUC lung/MIC of 51 correlated with a marked bactericidal, even eliminatory effect.

Tilmicosin

Although several successful artificial challenge studies have been carried out (Moore *et al.*, 1996; Paradis *et al.*, 2004; Nerland *et al.*, 2005) no MIC data for the challenge strains of *A. pleuropneumoniae* were available. The Morre *et al.*, (1996) results are shown in table 12. Shryock *et al.*, (2002) reported that there was good clinical efficacy with isolates up to 16µg/ml hence this was used as the clinical breakpoint but this could not be correlated to PK levels of tilmicosin in plasma, lung or macrophages.

Table 12 – Dose titration results 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	Ave lung score (%)	*A. pleuropneumoniae* recovery (%) day 14	ADG (g) day 0 to 14
0	100	35	0.41
100	65*	13*	0.63*
200	21**	8*	0.67**
300	10**	0*	0.71**
400	8**	0*	0.69**

*Key: - * p=0.05; ** p=0.05 from **

Tulathromycin

McKelvie *et al*., (2005) described the use of enrofloxacin as a positive control in a *M. hyopneumoniae* challenge study testing tulathromycin. Enrofloxacin was given at 5 mg/kg bodyweight (double recommended dose) for three days and tulathromycin was given at 2.5 mg/kg bodyweight as a single dose five to six 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 0.05 µg/ml but unfortunately, it was not recorded for enrofloxacin. (See table 13)

Table 13 – Comparison of tulathromycin PK/PD parameters and enrofloxacin for the treatment of enzootic pneumonia

Treatment	Lung lesion score (%)	MIC (µg/ml)	Cmax plasma/ MIC	Cmax lung/ MIC	AUC plasma/ MIC	AUC lung/ MIC
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 240 for tulathromycin against the *M. hyopneumoniae* isolate used. The resulting lung lesion reduction of 49% is typical 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 four times MIC was reported by Evans (2005) against *A. pleuropneumoniae*. By contrast the lung Cmax and AUC/MIC are exceptionally large, suggesting a lack of direct relationship as it was only an inhibitory effect. Enrofloxacin was used at double the normal dose and achieved a stronger mycoplasmacidal effect reducing lung lesions by 90%. A predicted MIC of ≤0.12 µg/ml, which is within normal MIC limits, could be estimated.

Hart *et al*., (2006) tested tulathromycin at 2.5 and 5.0 mg/kg as a single injection in pigs with ceftiofur Na at 3 mg/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 euthanased and the lung lesions scored. The MICs of tulathromycin and ceftiofur against the organism were 16 and 0.063µg/ml respectively (shown in tables 14 and 15.)

Table 14 – Comparative results of tulathromycin and ceftiofur for the treatment of A. pleuropneumoniae

Treatment	Deaths (%)	Lung lesions (%)	Weight gain (kg) Day 0 to 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 3mg/kg 3 days	0	10.0	4.52	56

Table 15 – Comparison of tulathromycin PK/PD parameters and ceftiofur for the treatment of A. pleuropneumoniae

Treatment	MIC (µg/ml)	Cmax plasma/ MIC	Cmax lung/ MIC	AUC plasma/ MIC	AUC lung/ MIC
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 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 with the product but that it is more related to AUC lung/MIC not AUC plasma or Cmax plasma. The arguments put forward by the authors were the MIC of 16 µg/ml was possibly overestimated (by four dilutions) due to culture method or the drug might concentrate in macrophages, like tilmicosin. In the first case the MIC would be nearer 1.0 µg/ml and the AUC plasma/MIC at a dose of 5 mg/kg bwt would be 24 and AUC lung/MIC would be 1229. If this were divided by 10 days duration of the study the plasma would be 2.4, which is very low and the lung would be 123, which is about the expected PK level. By comparison, ceftiofur, which gave a very strong inhibitory effect, especially in the first few days, which deteriorated later in the experiment, the Cmax/MIC and AUC/MIC were substantially over the recognised figures of 12 and 120 for plasma and AUC plasma/MIC.

The clinical effect was very good by day four (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 5 mg/kg dose and might be associated with an immune response to the challenge infection.

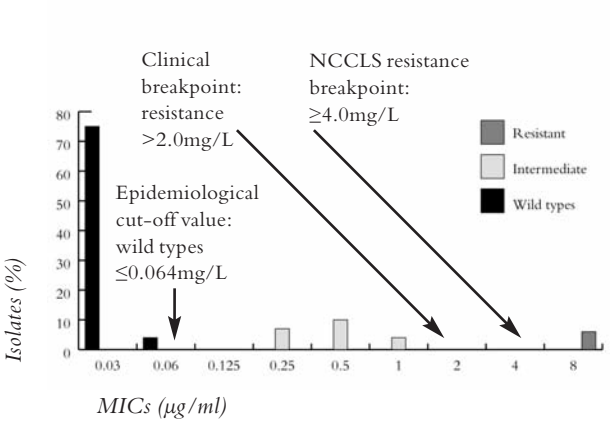
These examples show that lung concentrations and pharmacokinetics would appear to be important considerations in assessing the potential antibacterial effect of a substance, especially when the antimicrobial, such as tiamulin, tilmicosin and tulathromycin, concentrates in lung tissue. One theory to support this is when an organism such as *A. pleuropneumoniae* can cause an acute degree of necrosis in lung tissue and possibly disrupts the drug flow in and out of the cells. By contrast, *M. hyopneumoniae* is mainly surface dwelling and causes a comparatively mild and chronic infection and plasma/MIC concentrations correlate well with efficacy. Where antimicrobials do not concentrate in the lung to any degree, such as enrofloxacin and oxytetracycline, plasma/MIC levels appear to be quite satisfactory in determining PK/PD relationships and their efficacy. There are problems with the more fastidious bacteria, such as *A. pleuropneumoniae*, in determining the relevant MICs but less so for *P. multocida*. From the limited work carried out on the addition of serum to the culture media, the wrong MIC interpretation may be the cause of the discrepancies for tiamulin, tilmicosin and tulathromycin when we look at plasma/MIC relationships (Godinho *et al.*, 2005; Illambas *et al.*, 2008; Burch *et al.*, 2009). Mouton *et al.,* (2008) claim that it is unjustifiable to use tissue concentrations for PK/PD relationship assessments unless the organism lives intra-cellularly.

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

When antimicrobials are used they frequently leave behind a susceptibility/resistance pattern. The so called 'driver' for this selection is the antimicrobial concentration that is achieved in the target tissue of fluid. This also has a confirmatory effect that the antimicrobial is reaching that level when reliable PK data is lacking.

Bywater *et al.*, (2006) described this novel approach to demonstrate antimicrobial susceptibility/resistance development. This method also helps differentiate and establish the epidemiological cut-off value, the natural susceptibility pattern seen before exposure to an antimicrobial. This may or may not correlate with the clinical breakpoint based on antimicrobial PK results and the microbiological breakpoint for resistance, which may be different again (see Figure 6). They used ciprofloxacin and *E. coli* as an example and there is a double peak after the epidemiological breakpoint where two stepwise mutations have taken place, which lead to reduced susceptibility and eventually to complete resistance.

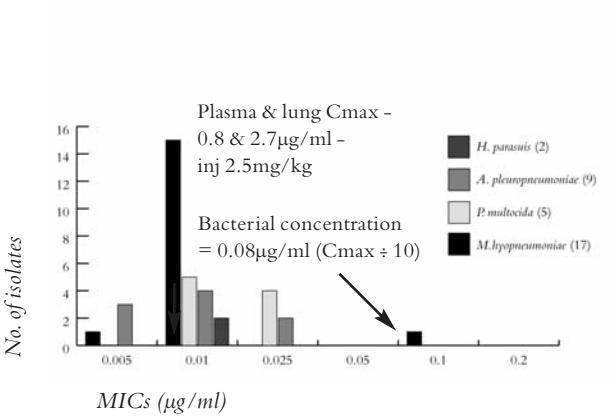
Figure 6 – Antimicrobial sensitivity patterns to determine epidemiological, clinical and microbiological resistance breakpoints, (based on ciprofloxacin and E. coli)



Enrofloxacin

When the major porcine respiratory bacteria and mycoplasma are graphed out for enrofloxacin (See fig. 7) in this early work by Hannan *et al.*, (1989) the majority of isolates are below the Cmax plasma divided by 10 figure (0.08 µg/ml) suggesting that the majority of these would be treated effectively (see Figure7.) and the bacteria would likely be killed. Intermediate effects, such as bactericidal or inhibitory effects, could be expected up to 0.8µg/ml but elimination is unlikely to occur at these higher MIC levels.

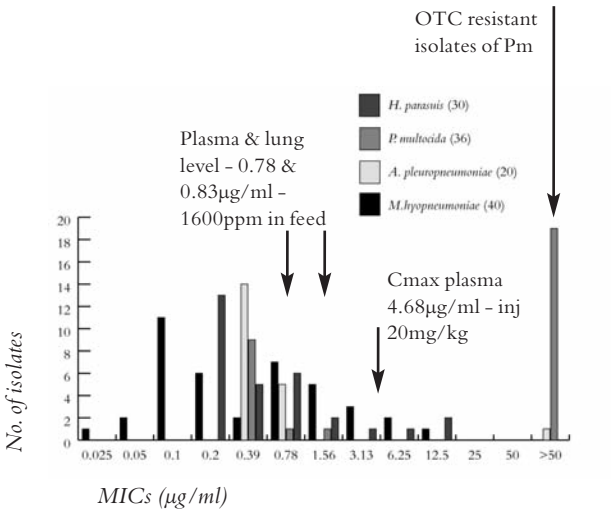
Figure 7 – Susceptibility pattern for enrofloxacin against common respiratory pathogens



Oxytetracycline

There are two peaks for *M. hyopneumoniae*, suggesting some mutation at about 0.39 µg/ml and the next is at about 1.56 µg/ml, which is the epidemiological breakpoint for the respiratory bacteria. There is then a major shift to >50 where there is true resistance (see Figure 8).

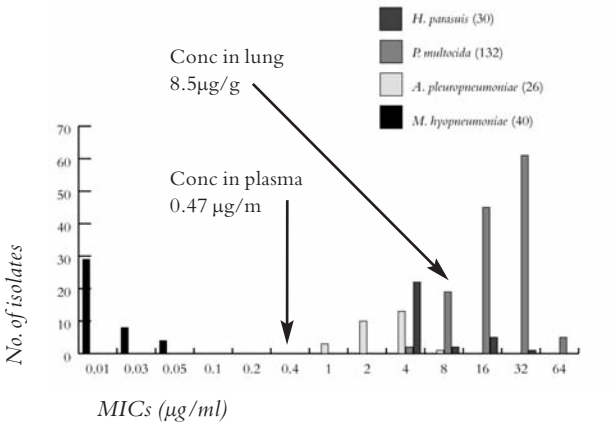
Figure 8 – Susceptibility pattern for oxytetracycline against common respiratory pathogens (Inamoto et al., 1994; Pijpers et al., 1990; Martin-de la Fuente et al., 2007)



Tiamulin

Tiamulin shows a markedly different susceptibility pattern with *M. hyopneumoniae* showing an epidemiological breakpoint at 0.05 µg/ml and the next one is about 8.0 µg/ml for *A. pleuropneumoniae* and *H. parasuis*. This fits in well with the clinical trial results with tiamulin given in the drinking water at 180ppm and the plasma and lung concentrations. *Pasteurella multocida* generally seem to be not susceptible at MICs above 8.0 µg/ml (see Figure 9).

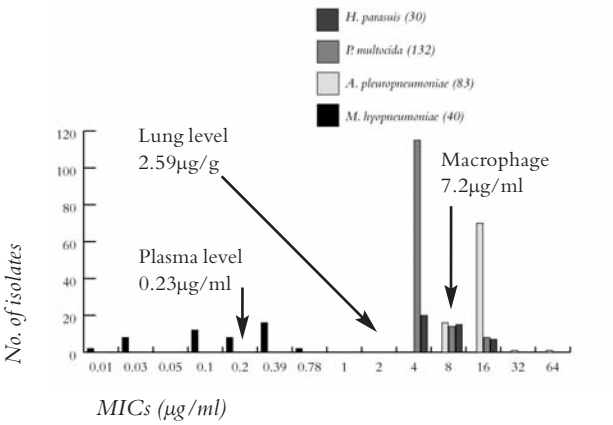
Figure 9 – Susceptibility pattern for tiamulin against common respiratory pathogens (Inamoto et al., 1994; Vera-Lizarazo et al., 2006; Casals et al., 1990; Martin-de la Fuente et al., 2007)



Tilmicosin

Tilmicosin seems to have the split MIC pattern similar to tiamulin, *e.g.* one for *M. hyopneumoniae*, which more coincides with plasma levels and a second one for respiratory bacteria, (see fig. 10) which has an epidemiological breakpoint and clinical breakpoint associated with the macrophage concentration around 8.0 µg/ml. The lung concentration seems remarkably low in comparison with the bacterial MICs, which lends weight to the macrophage argument. Artificial challenge studies with known MIC bacteria would be helpful to clarify this.

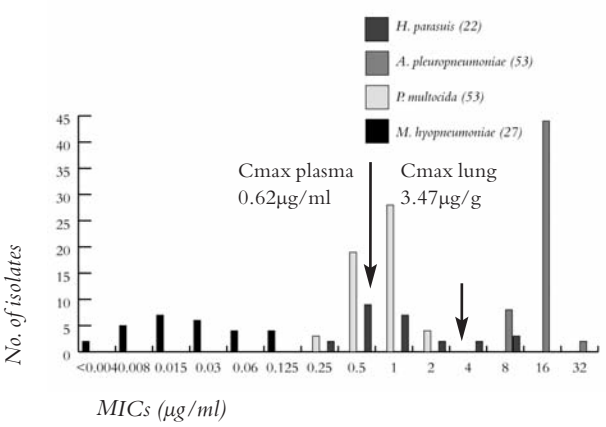
Figure 10 – Susceptibility pattern for tilmicosin against common respiratory pathogens (Inamoto et al., 1994; Matter et al., 2007; Vera-Lizarazo et al., 2006; Martin-de la Fuente et al., 2007)



Tulathromycin

Has three separate peaks, one associated with *M. hyopneumoniae*, the next associated with the non-fastidious *P. multocida* and *H. parasuis*. These two peaks correlate well with plasma and lung levels. The third peak is for *A. pleuropneumoniae* (see figure 11). This does not correlate with lung levels, yet clinical efficacy has been observed. This is likely to be due to difficulties in determining the relevant MICs for *A. pleuropneumoniae*, as there appears to be a lot of variation in assessment. It may be associated with higher macrophage levels, like tilmicosin but probably less likely as the macrophage concentrations are much lower than AUC lung/plasma ratio, like tiamulin. There is not enough MIC data to demonstrate resistance patterns to tulathromycin to confirm this except for *H. parasuis*, which appears to dip at 4.0 µg/ml.

Figure 11 – Susceptibility pattern for tulathromycin against common respiratory pathogens (Godhino et al., 2005)



Conclusion

Our understanding of PK/PD relationships of antimicrobials and their clinical effects are still developing as more information is made available. Treatment of *M. hyopneumoniae* appears to correlate well with plasma concentrations. Good pharmacokinetic data is essential for both plasma and lung. Where there is little difference between the two it is less important for respiratory infections and plasma PK relationships are suitable. For those compounds that concentrate in lung tissue there would appear to be a significant relationship between lung PK and efficacy. The role of antibiotic concentrations in neutrophils and alveolar macrophages would also appear to be significant against bacterial infections, especially for tilmicosin (see Table 16) where the concentrations markedly exceed lung concentrations. Tiamulin's estimated leucocyte concentration is almost identical to lung concentration and tulathromycin's are both similar.

Care must be taken to include all bioactive metabolites, so sometimes it is better to use a microbiological assay rather than HPLC, especially when the antimicrobial is extensively metabolised, such as tiamulin, or the major metabolite is equally active in the case of enrofloxacin and ciprofloxacin.

The pharmacodynamics of some fastidious bacteria, such as *A. pleuropneumoniae*, also play a confusing role as their MICs can be quite variable according to the method used. The NCCLS/CLSI method appears to give very high MICs. The addition of serum to the medium or culture in pure serum can have a major effect on MIC determination. This area needs to be further explored. Efficacy can only be confirmed by good quality artificial challenge studies with known MIC bacteria and this is the basis of the NCCLS/CLSI interpretation of sensitivity. The less fastidious bacterium, *P. multocida* and also *H. parasuis* do not seem to pose this problem to the same extent and can help to give a lead to effective PK/PD activity, as determined by epidemiological cut off values.

These susceptibility/resistance patterns require that the antimicrobial had to be available for some time to allow for resistance development to occur and suitably large numbers of isolates need to be tested.

For those antimicrobials that accumulate in lung tissue and have a high lung/plasma ratio, (lung pharmacokinetics) it is very tempting to assume that they can have a significant role in establishing PK/PD relationships and clinical efficacy against bacterial respiratory infections. The role of alveolar macrophage concentrations also would appear to have an effect, when the antimicrobial concentrates in them to a very high degree, such as tilmicosin. However, it would appear that these may be false pharmacokinetic parameters, according to the clinical pharmacologists, and that only free drugs in serum is the best surrogate. This would indicate that the pharmacodynamic (MIC) data

Table 16 – Comparison of plasma, leucocyte and lung concentrations for tilmicosin, tiamulin and tulathromycin and ECOVs

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

Key – PMNs = polymorphonucleocytes; AMs = alveolar macrophages

used is incorrect. Further work is required to look at the impact of serum in culture media on MICs to resolve these anomalies.

References

Aarestrup, F.M. and Jensen N.E. (1999) Susceptibility testing of *Actinobacillus pleuropneumoniae* in Denmark. Evaluation of three different media of MIC-determinations and tablet diffusion tests. Veterinary Microbiology, **64**, 299-305.

Aarestrup, F.M., Seyfarth, A.M. and Angen, O. (2004) Antimicrobial susceptibility of *Haemophilus parasuis* and *Histophilus somni* from pigs and cattle in Denmark. Veterinary Microbiology, **101**, 143-146.

Anderson, M.D., Stroh, S.L. and Rogers, S. (1994) Tiamulin (Denagard®) activity in certain swine tissues following oral and intramuscular administration. Proceedings of the American Association of Swine Practitioners, Chicago, Illinois, USA, 115-118.

Asanuma, K., Shimazaki, S. and Hirata, K. (1986) A study concerning the distribution of OTC and CTC in the lung and blood of pigs. Journal of Animal Drugs, **6**, 9, 1-14.

Banting, A., De, L. and Baggot, J.D. (1996) Comparison of the pharmacokinetics and local tolerance of three injectable oxytetracycline formulations in pigs. Journal of Veterinary Pharmacology and Therapeutics, **19**, 50-55.

Benchaoui, H.A., Nowakowski, M., Sherington, J., Rowan, T.G. and Sunderland, S.J. (2004) Pharmacokinetics and lung concentrations of tulathromycin in swine. Journal of Veterinary Pharmacology and Therapeutics, **27**, 203-210.

Blais, J. and Chamberland, S. Intracellular accumulation of tilmicosin in primary swine alveolar macrophages. Proceedings of the 13th International Pig Veterinary Society Congress, Bangkok, Thailand, p. 331.

Brown, S.A., Hanson, B.J., Mignot, A., Millerioux, L., Hamlow, P.J., Hubbard, V.L., Callahan, J.K. and Kausche, F.M. (1999) Comparison of plasma pharmacokinetics and bioavailability of ceftiofur sodium and ceftiofur hydrochloride in pigs after a single intramuscular injection. Journal of Veterinary Pharmacology and Therapeutics, **22**, 35-40

Burch, D.G.S. (2004) The comparative efficacy of antimicrobials for the prevention and treatment of enzootic pneumonia and some of their pharmacokinetic and pharmacodynamic relationships. The Pig Journal, **53**, 8-27.

Burch, D.G.S. and Klein, U. (2008) Pharmacokinetic/pharmacodynamic relationships of tiamulin (Denagard ® for respiratory infections in pigs. Proceedings of the 20th International Pig Veterinary Society Congress, Durban, S. Africa, **2**, p. 494.

Burch, D.G.S., Pridmore, A. and Klein, U. (2009) PK/PD integration of tiamulin and *Actinobacillus pleuropneumoniae*. Journal of Veterinary Pharmacology and Therapeutics (Supplement 1), In press.

Bywater, R., Silley, P. and Simjee, S. (2006) Letter: Antimicrobial breakpoints – definitions and conflicting requirements. Veterinary Microbiology, **118**, 158-159.

Casals, J.B., Nielsen, R. and Szancer, J. (1990) Standardisation of tiamulin for routine sensitivity of *Actinobacillus (Haemophilus) pleuropneumoniae* (Ap). Proceedings of the 11th International Pig Veterinary Society Congress, Lausanne, Switzerland, p. 43.

Chang, F.C., Chang, L.C, Chang, Y.F., Chen, M. and Chiang, T.S. (2002) Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae*, *Escherichia coli* and *Salmonella choleraesuis* recovered from Taiwanese swine. Journal of Veterinary Diagnostic Investigation, **14**, 153-157.

DeGeeter, M.J., Barbiers, A.R. and Stahl, G.L. (1980) Concentration of lincomycin in body tissues and fluids of swine fed diets fortified with the antibiotic. Proceedings of the International Pig Veterinary Society Congress, Copenhagen, Denmark, p. 283.

Evans, N.A. (2005) Tulathromycin: an overview of a new triamilide antimicrobial for livestock respiratory disease. Veterinary Therapeutics, **6**, 2, 83-95.

Godinho, K.S., Keane, S.G., Nanjiani, I.A., Benchaoui, H.A., Sunderland, S.J., Jones, M.A., Weatherley, A.J., Gootz, T.D. and Rowan, T.G. (2005) Minimum concentrations of tulathromycin against respiratory bacterial pathogens isolated from clinical cases in European cattle and swine and variability arising from changes in *in vitro* methodology. Veterinary Therapeutics, **6**, 2, 113-121.

Gutierrez-Martin, C.B., del Blanco, N.G., Blanco, M., Navas, J. and Rodriguez-Ferri, E.F. (2006) Changes in antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs in Spain during the last decade. Veterinary Microbiology, **115**, 218-222.

Hannan, P.C.T., Bhogal, B.S. and Fish, J.P. (1982) Tylosin Tartrate and tiamutilin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenate containing mycoplasmas, bacteria and viruses. Research in Veterinary Science, **33**, 76-88.

Hannan, P.C.T., O'Hanlon, P.J. and Rogers, N.H. (1989) *In vitro* evaluation of various quinolone antibacterial agents against veterinary mycoplasmas and porcine respiratory bacterial pathogens. Research in Veterinary Science, **46**, 202-211.

Hart, F.J., Kilgore, R.W., Meinert, T.R., Nutsch, R.G., Sunderland, S.J. and Lechtenberg, K.F. (2006) Efficacy of tulathromycin in the treatment of respiratory disease in pigs caused by *Actinobacillus pleuropneumoniae*. Veterinary Record, **158**, 433-436.

Hoffmann, H., Hartl, A., Horn, U., Kielstein, P., Drewello, J., Fossberg, W. and Seupel, B. (1983) Antimicrobial activity in tissue of swine, following subcutaneous administration of turimycin. Archive of Experimental Veterinary Medicine, **37**, 599-607.

Ibayashi, T., Okada, M. and Ando, N. (1994) Pulmonary concentrations of lincomycin, tylosin, acetylisovaleryltylosin and tiamulin given as feed additives in swine. Proceedings of the 13th International Pig Veterinary Society Congress, Bangkok, Thailand, p. 354.

Illambas, J.M., Potter, T., Rycroft, A. and Lees P. (2008) Pharmacodynamics of tulathromycin *in vitro* and ex vivo against calf pathogens. Proceedings of the 4th International Conference on Antimicrobial Agents in Veterinary Medicine, Prague, Czech Republic, p. 93.

Inamoto, T., Takahashi, H., Yamamoto, K., Nakai, Y. and Ogimoto, K. 1994) Antibiotic susceptibility of *Mycoplasma hyopneumoniae* isolated from swine. Journal of Veterinary Medical Sciences, **56**, 2, 393-394.

Jacobson, M., Franklin, A. and Fellstrom, C. (1994) Comparison of blood and lung levels of antibiotics in swine given Aureomycin vet (Chlortetracycline) or Terramycin vet (oxytetracycline) in the feed. Proceedings of the 13th International Pig Veterinary Society Congress, Bangkok, Thailand, p. 358.

Martin de la Fuente, A.J., Tucker, A.W., Navas, J., Blanco, M., Morris, S.J. and Gutierrez-Martin, C.B. (2007) Antimicrobial susceptibility patterns of *Haemophilus parasuis* from pigs in the United Kingdom and Spain. Veterinary Microbiology, **120**, 184-191

Matter, D., Rossano, A., Limat, S., Vorlet-Fawer, L., Brodard, I. and Perreten, V. (2007) Antimicrobial resistance profile of *Actinobacillus pleuropneumoniae* and *Actinobacillus porcitonsillarum*. Veterinary Microbiology, **122**, 146-156.

McKellar, Q., Escala, J. and Szancer, J. (2004) Plasma and tissue kinetic study of tiamulin (Tiamutin) in pigs. Proceedings of the 18th International Pig Veterinary Society Congress, Hamburg, Germany, **2**, p. 622.

McKelvie, J., Morgan, J.H., Nanjiani, I.A., Sherington, J., Rowan, T.G. and Sunderland, S.J. (2005) Evaluation of tulathromycin for the treatment of pneumonia following experimental infection of swine with *Mycoplasma hyopneumoniae*. Veterinary Therapeutics, **6**, 2, 197-202.

Moore, G.M., Mowrey, D.H., Tonkinson, L.V., Lechtenberg, K.F. and Schneider, J.H. (1996) Efficacy of dose determination study of tilmicosin phosphate in feed for control of pneumonia caused by *Actinobacillus pleuropneumoniae* in swine. American Journal of Veterinary Research, **57**, 2, 220-223.

Mouton, J.W., Theuretzbacher, U., Craig, W.A., Tulkens, P.M., Derendorf, H. and Cars, O. (2008) Tissue concentrations: do we ever learn? Journal of Antimicrobial Chemotherapy, **16**, 235-237.

Nerland, E.M., LeBlanc, J.M., Fedwick, J.P., Morck, D.W., Merrill, J.K., Dick. P., Paradis, M-A. and Buret, A.G. (2005) Effects of oral administration of tilmicosin on pulmonary inflammation in piglets experimentally infected with *Actinobacillus pleuropneumoniae*. American Journal of Veterinary Research, **66**, 1, 100-107.

Nielsen, B.H. and Szancer, J. (1998) Uptake and intracellular concentration of tiamulin in human polymorphonuclear leukocytes compared with norfloxacin. Proceedings of the 15th International Pig Veterinary Society Congress, Birmingham, UK, **3**, p. 241.

NOAH (2007) Compendium of Datasheets for Animal Medicines 2008. National Office of Animal Health Ltd, Enfield, Middlesex, UK.

Paradis, M-A., Vessie, G.H., Merrill, J.K., Dick, C.P., Moore, C., Charbonneau, G., Gottschalk, M., Macinnes, J.I., Higgins, R., Mittal, K.R., Girard, C., Aramini, J.J. and Wilson, J.B. (2004) Efficacy of tilmicosin in the control of experimentally induced *Actinobacillus pleuropneumoniae* infection in swine. Canadian Journal of Veterinary Research, **68**, 7-11.

Pijpers, A., van Klingeren, B., Schoevers, E.J., Verheiden, J.H.M. and van Miert, A.S.J.P.A.M. (1989) *In vitro* activity of five tetracyclines and some other antimicrobial agents against four porcine respiratory tract pathogens. Journal of Veterinary Pharmacology and Therapeutics, **12**, 267-276.

Pijpers, A., Schoevers, E.J., Haagsma, N. and Verheijden, J.H.M. (1990a) Chapter 6: 'Plasma levels of oxytetracycline, doxycycline and minocycline in pigs after oral administration in feed'. In Doctoral Thesis, University of Utrecht, 85-103.

Pijpers, A., Schoevers, E.J., van Leengoed, L.A.M.G., Koeman, J., Vernooy, J.C.M. and Verheijden, J.H.M. (1990b) Chapter 7: 'Prophylaxis of pleuropneumonia by oxytetracycline in feed medication in pigs. In Doctoral Thesis, University of Utrecht, 105-122.

Scheer, M. (1987) Concentrations of active ingredient in the serum and in tissues after oral and parenteral administration of Baytril. Veterinary Medical Review, **2**, 104-118.

Shryock, T.R., Staples, J.M. and DeRosa, D.C. (2002) Minimum inhibitory concentration breakpoints and disk diffusion inhibitory zone interpretative criteria for tilmicosin susceptibility testing against *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* associated with porcine respiratory disease. Journal of Veterinary Diagnostic Investigation, **14**, 389-395.

Sidoli, L., Barigazzi, G., Schianchi, P., Russell, C.H. and Russell, S.B. (1984) Minimum inhibitory concentrations of antibacterial agents against strains of *Haemophilus pleuropneumoniae* from swine. Veterinary Medicine, 703-705.

Siegel, D., Earley, L., Smothers, C.D., Sun, F. and Ricketts, A.P. (2004) Cellular uptake of the triamilide tulathromycin by bovine and porcine phagocytic cells *in vitro*. Journal of Animal Science, **82**, Supplement 1, p. 186.

Smith, I.M., Mackie, A. and Lida, J. (1991) Effect of giving enrofloxacin in the diet to pigs experimentally infected with *Actinobacillus pleuropneumoniae*. Veterinary Record, **129**, 25-29.

Stoker, J., Parker, R. and Spencer, Y. (1996) The concentration of tilmicosin in pig serum and respiratory tissue following oral administration with Pulmotil® via the feed at a level of 400g/tonne. Proceedings of the 14th International Pig Veterinary Society Congress, Bologna, Italy, p. 656.

Swenson, G.H. and Barbiers, A.R. (1976) The distribution and depletion of lincomycin in swine following parenteral administration. Proceedings of the 4th International Pig Veterinary Society Congress, Ames, Iowa, USA, B.5.

Thomson, T.D., Darby, J.M., Moran, J.W. and Tonkinson, L.V. (1994) Serum and lung tilmicosin concentration in swine following dosing with tilmicosin fortified feed. Proceedings of the 13th International Pig Veterinary Society Congress, Bangkok, Thailand, p. 330.

Toutain, P. (2003) Pharmacokinetics /pharmacodynamics integration in dosage regimen optimization for veterinary medicine. Journal of Veterinary Pharmacology and Therapy, 26 (Supplement 1) 1-8.

Vera-Lizarazo, Y.A., Rodriguez-Ferri, E.F., Martin-de la Fuente, A.J. and Gutierrez-Martin, C.B. (2006) Evaluation of changes in antimicrobial susceptibility patterns of *Pasteurella multocida* subsp *multocida* isolates from pigs in Spain 1987-1988 and 2003-2004. American Journal of Veterinary Research, **67**, 4, 663-668.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A. and Haesebrouck, F. (2004) *In vitro* susceptibilities of *Mycoplasma hyopneumoniae* field isolates. Antimicrobial Agents and Chemotherapy, **48**, 11, 4470-4472.