

# THE RATIONAL USE OF ANTIBIOTICS AND IN RELATION TO ANTIBIOTIC RESISTANCE

DAVID G S BURCH

*Octagon Services Ltd, Old Windsor, Berkshire, UK*

**Key words:** Antibiotics, Resistance, Swine

**Summary:** When antimicrobials are used there is always a risk that the selection of antimicrobial resistance will occur. Some bacterial species appear to develop resistance more quickly than others, such as *Escherichia coli* and some antimicrobials have resistance developed quite easily against them e.g. the tetracyclines. The first approach is to avoid or reduce the use of antimicrobials by using high health (specific pathogen free (SPF) animals, employing disease eradication or vaccination programmes where possible and using management and hygiene strategies to reduce infectious challenge. When forced to use antimicrobials, optimum programmes of use must be employed to reduce the opportunities for resistance development. Making sure the right organism is being treated, by culturing and antimicrobial susceptibility testing, using knowledge of effective drug concentrations to kill the organism in the target tissues or fluids. Understanding the pharmacokinetics of the antimicrobials is essential and relating these to the pharmacodynamic effect on the bacteria to achieve not only a clinical cure but also a bacterial cure. Going beyond the mutant selection window, also helps to improve the long term clinical effect of an antimicrobial and reduces the development of resistance. This paper looks at strategies to help reduce resistance development in pig production by the use of pharmacokinetic/pharmacodynamic integration and application.

## INTRODUCTION

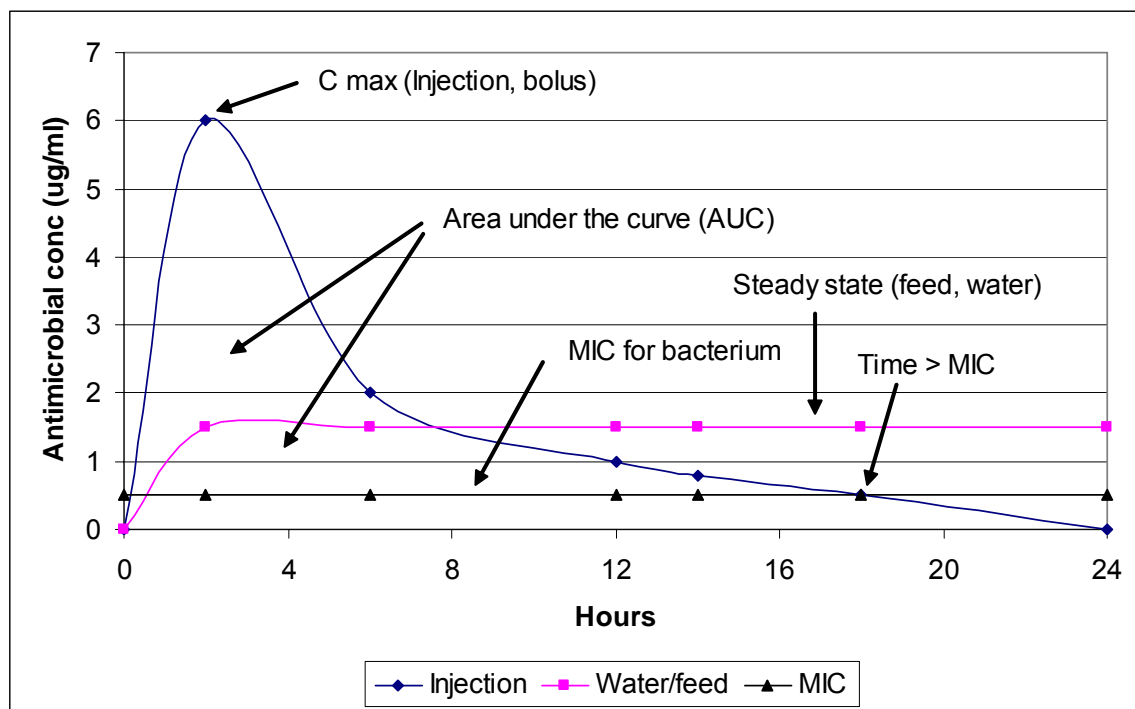
When antimicrobials are used there is always a risk that the selection of antimicrobial resistance will occur. In the UK, recent figures have shown (VMD, 2008) that 387 tonnes of antimicrobial drugs were used in 2007 in veterinary medicine, both for food producing animals and for companion animals. Eighty nine per cent was given via the oral route, mainly in feed premixes or drinking water medication and only 10% by injection. Tetracyclines were the most commonly used antibiotic (45%) followed by trimethoprim/sulphonamide combinations (19%) and beta-lactam antibiotics (penicillins, synthetic penicillins etc) at 17%. The cephalosporins and fluoroquinolones, which are considered of critical importance in human medicine only accounted for 1.6% and 0.5% of animal use respectively, which is low. Use in swine, although only 9 million are slaughtered each year in the UK, is estimated to account for 53% of antimicrobial use. As a result, there is a marked responsibility on the swine veterinarian to use antimicrobial drugs responsibly and judiciously to achieve the best outcomes both clinically, bacteriologically and economically for his clients, to try to limit the development of antimicrobial resistance in the pig population but also to try to reduce the chances of spread of resistance to the human population.

This paper will review the understanding of pharmacokinetics (PK) in pigs, the pharmacodynamic (PD) information that may be developed in clinical and university laboratories and how we can integrate the two, so that the use, dose and route of antibiotic and the possibilities of resistance development avoidance can also be included in the decision making processes for antimicrobial use.

## BASIC PK/PD RELATIONSHIPS

### a. Pharmacokinetic aspects

A basic understanding of the pharmacokinetics of drugs is essential to understand how they work in the pig (Fig.1)

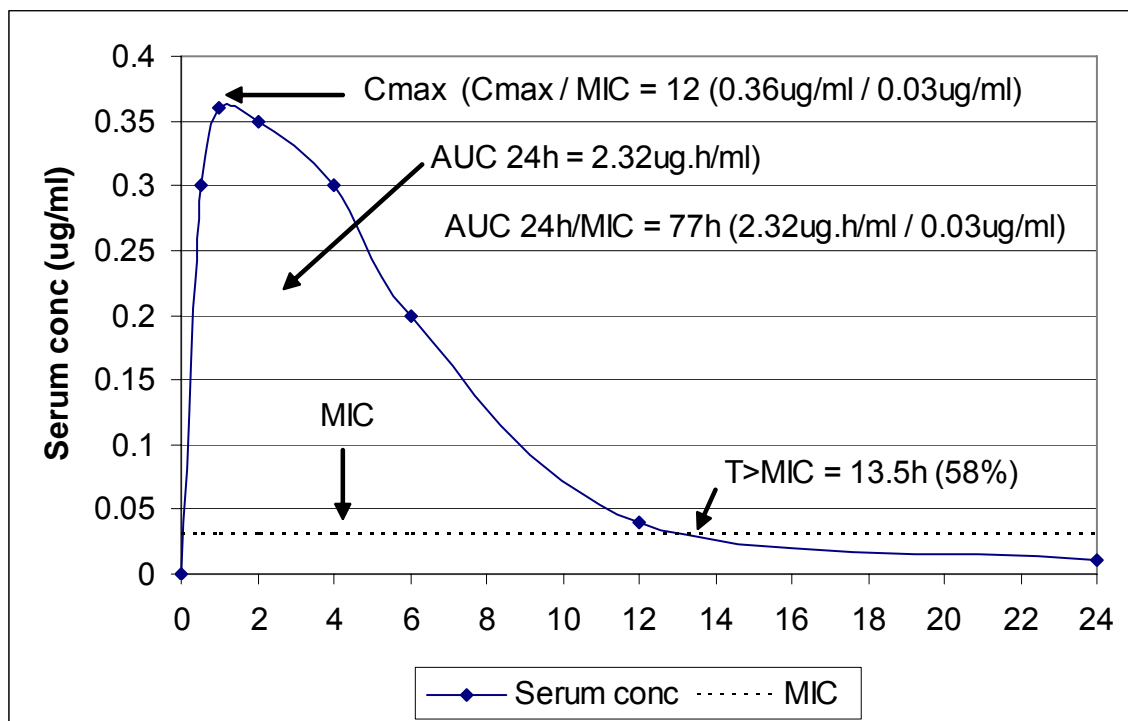


**Fig. 1.** Comparison of the basic pharmacokinetics of an antibiotic given by injection or orally in feed or water

The maximum concentration ( $C_{max}$ ) is the highest plasma concentration recorded following administration. Usually, when administered by injection it gives a high peak, which then tails off. The time it reaches the peak concentration is known as the  $T_{max}$ . For oral medication given in feed or water the peaks are usually much lower and the  $T_{max}$  is longer but if they are on *ad libitum* feeding, the plasma levels can be quite steady during the day. The concentration curves can also be measured and the area under the curve over 24 hours ( $AUC_{24h}$ ) is also a useful measurement. The decline of the curve, when compared with the minimum inhibitory concentration (MIC) also gives the time the curve is above the MIC ( $T > MIC$ ), which is also useful especially for determining dosage interval i.e. should a drug be given once or twice a day etc.

The C<sub>max</sub> relationship with MIC is important for some antimicrobial drugs like the aminoglycosides (apramycin, gentamicin etc) and also the fluoroquinolones (enrofloxacin, marbofloxacin etc) as it has been shown (Schentag, 2000) that when the C<sub>max</sub>/MIC ratio is approximately 10-12, the bacterium was successfully killed and clinical outcomes were improved in immuno-compromised patients in hospitals. The MIC is similar to the minimum bactericidal concentration (MBC) for these antimicrobial drugs and the MBC/MIC ratio = 1-2: 1. They also show a good post-antibiotic effect (PAE) i.e. the bacterium does not start to re-grow for some time after removal of the antibiotic. Colistin, a polymixin, also falls into this category.

The AUC 24h is also a useful PK parameter for bactericidal drugs as it involves both a time and concentration component, especially for injectables when there is such a fluctuation in concentrations. The AUC 24h/MIC ratio of about 100-120 also gives a useful prediction for bacterial kill for the bactericidal antimicrobial drugs such as the aminoglycosides and the fluorquinolones, trimethoprim/sulphonamide combinations and the beta lactams (penicillins and cephalosporins), which also appear to fit in with this relationship. Time >MIC is often considered to be of more significance for the beta lactam antibiotics (Lees et al, 2008) but there is also an increased killing rate with higher concentrations. Antibiotics that are time and concentration dependent are called co-dependent and most antibiotics in swine medicine have this relationship (Fig 2).



**Fig. 2.** PK/PD integration relationship example for enrofloxacin and *Pasteurella multocida* (MIC 0.03µg/ml) (Lees & Aliabadi, 2002)

If the AUC 24h/MIC ratio is divided by 24 hours it is approximately 4-5times the MIC and is another useful relationship for less fluctuating antimicrobial concentrations found

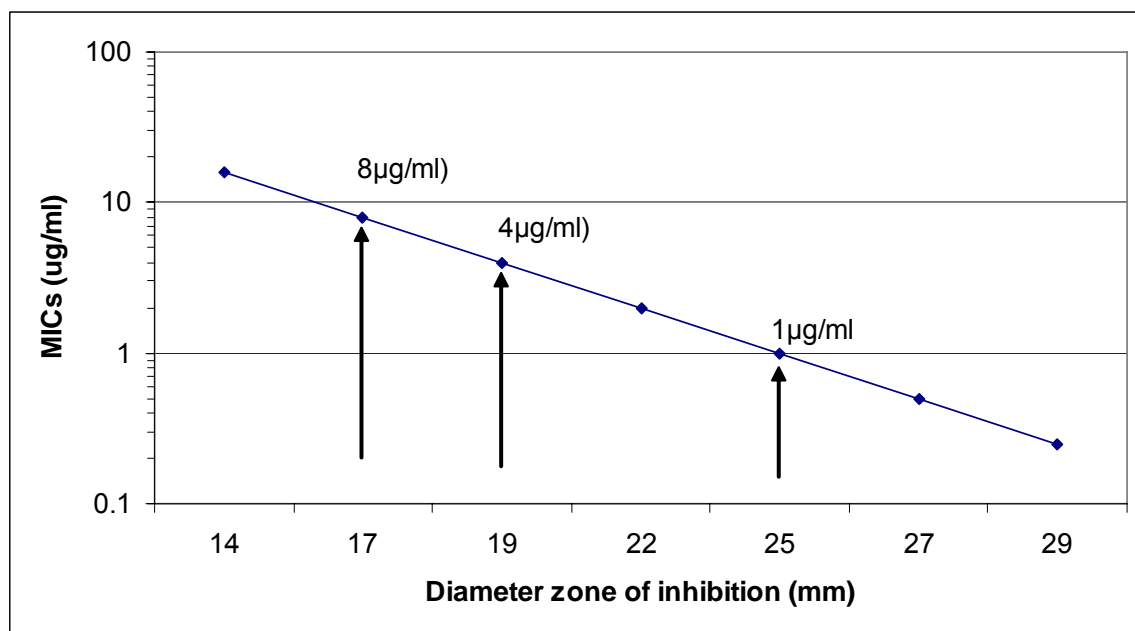
in plasma or gut contents after oral medication. These concentrations are almost steady-state (SS) concentrations and are therefore useful for cross reference to single time-point concentrations such as those in the lung.

For bacteriostatic antibiotics, such as the tetracyclines, macrolides, lincosamides and pleuromutilins, the AUC 24h/MIC gives a reference point for inhibition or a bacteriostatic effect, e.g. an AUC 24h/MIC = 24h. However, to achieve a bactericidal effect relationship, then the AUC 24h needs to be divided by the MBC which can be substantially different from the MIC e.g. MBC/MIC ratio = 2-40: 1.

### **b. Pharmacodynamic aspects - antimicrobial susceptibility testing**

There are a variety of methods to determine the susceptibility of an organism to an antibiotic. The Clinical and Laboratories Standards Institute (CLSI – formerly NCCLS) is endeavoring to establish set procedures that can be reproduced by different laboratories around the world and unify standards. Many laboratories have their own methods of culture and susceptibility testing for bacteria and these can give different results, so a standardised approach has been developed but the process is on-going and not yet finalised for all veterinary bacteria or veterinary antimicrobial drugs.

One of the common methods used in clinical veterinary laboratories is the Kirby-Bauer Method using sensitivity discs containing a known strength of antibiotic placed on a culture plate with the organism growing. Sensitivity is judged visually on the size of the zone of inhibition, which indicates approximately if it is susceptible, intermediate or totally resistant, however, a relatively simple technique of measuring the zone can also give more information on the MIC of the organism by relating it to graphs, comparing zone sizes with MICs (Fig. 3). This is part of the CLSI work also.



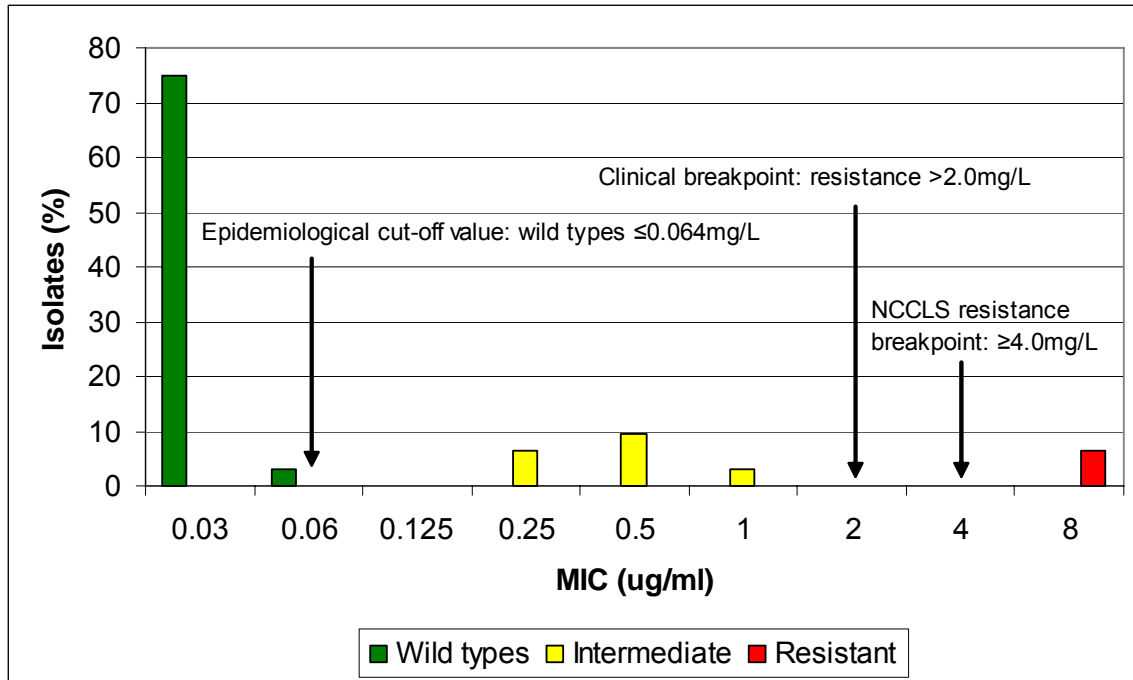
**Fig.3.** Diameter of zone of inhibition and MIC for tiamulin and *A. pleuropneumoniae* (Casals et al, 1990)

Minimum inhibitory concentrations are determined by commonly using doubling dilutions of antibiotics to determine the concentration that inhibits growth of the organism. This can be carried out in test tubes with broth, micro-tubes in plates, agar plates containing the antibiotic. There are some variations and inaccuracies, such as doubling the dilution means there is a 50% variation between each dilution. This can be overcome by using over-lapping concentrations but this is not routinely done. More recently, further limitations have been highlighted by the standard culture techniques used that they do not always represent the growth of the organism in plasma or similar extracellular fluids in the body where other factors, such as proteins, may have a role. This is one of the weaknesses of the CLSI system for some respiratory pathogens such as *A. pleuropneumoniae*, which have recently come to light and which have made it impossible to carry out PK/PD integration reliably for some antibiotics such as tulathromycin, tilmicosin and tiamulin, where MICs far exceed plasma concentrations.

### **c. Susceptibility patterns and breakpoints**

When a number of MICs of an antibiotic have been established for a bacterial species, the MIC 50, MIC 90 and range are often described. The MIC 50 and MIC 90 are the antibiotic concentrations, which inhibit 50% and 90% of the different isolates cultured, respectively and the range is the minimum and maximum concentration. These are useful to give an overall idea of susceptibility to an antibiotic, but they need to be put into context in relation to the antimicrobial concentration that can be achieved either in plasma or in gut contents for example to determine what are the clinical breakpoints for a bacterium and antibiotic.

Bywater et al (2006) described the new concept of epidemiological cut-off value (ECOV) or 'wild type' breakpoint for bacterial populations that have not been exposed to antibiotics and compared this with the clinical breakpoint where drug concentrations can reach and also the microbiological breakpoint, which may be different and even higher (Fig. 4). They used ciprofloxacin and *E. coli* as an example.

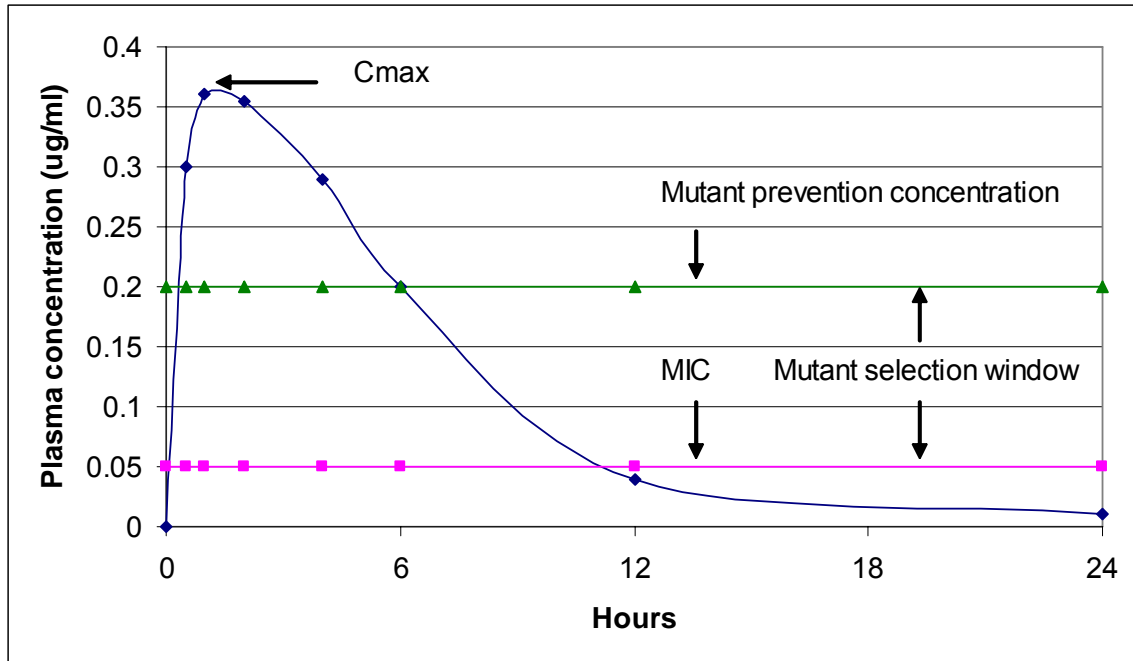


**Fig.4.** New concepts – different breakpoint determinations (Bywater et al, 2006)

There is a distinct first cluster of isolates, which represents the wild types. The second cluster represents the first mutant stage, which is nalidixic acid resistant, but still susceptible to high levels of ciprofloxacin and the third cluster are considered truly resistant after a second mutation.

Some antibiotics have a one step resistance development like nalidixic acid, and thus the susceptibility pattern can indicate how resistance develops and also more accurately the percentage of isolates that remain sensitive or have developed resistance, much more easily than the MIC 50 or 90.

Mutations in bacteria occur spontaneously at rates of  $10^6$  and  $10^{10}$  per gene generation. Drlica (2003) described the concept of using antibiotics at concentrations above the first mutant stage, so that it would kill the wild types and the first stage mutants based on the unlikely chances that a double mutation would occur. The concepts of the mutation selection window and mutation prevention concentration (MPC) appear to offer a very effective approach to reducing resistance development for some antibiotics (Fig. 5).



**Fig. 5.** Diagram of mutant selection window and mutant prevention concentration in relation to MIC

An example of this in veterinary medicine is enrofloxacin against *E. coli* infections in piglets. Wiuff et al (2002) measured the concentrations of enrofloxacin in plasma and in gut contents after oral dosing piglets with 2.5mg/kg bodyweight. When this is compared with characteristic MIC susceptibility patterns of *E. coli*, one can see the concentrations in the jejunal contents would not only kill the wild types but also the first stage mutants (Fig. 6).

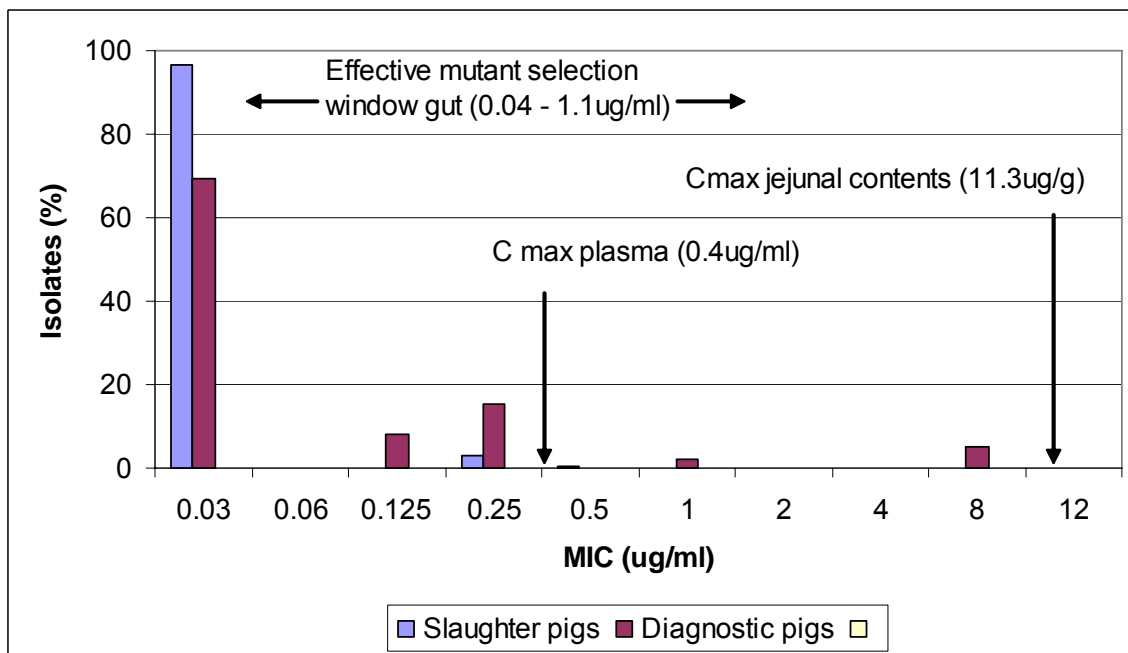


Fig. 6. Mutant selection window for enrofloxacin in jejunal contents against *E.coli* in pigs after oral dosing

The greater the concentration is above the MPC the less likely a mutant can overgrow in a population, as it is inhibited or killed. Mutant enrichment occurs above the MIC i.e. in the mutant selection window, but not below the MIC.

Tiamulin concentrations in the colon, when used at high levels to treat swine dysentery (Anderson et al, 1994), also exceed the first mutation step concentrations of *Brachyspira hyodysenteriae* (Fig. 7).

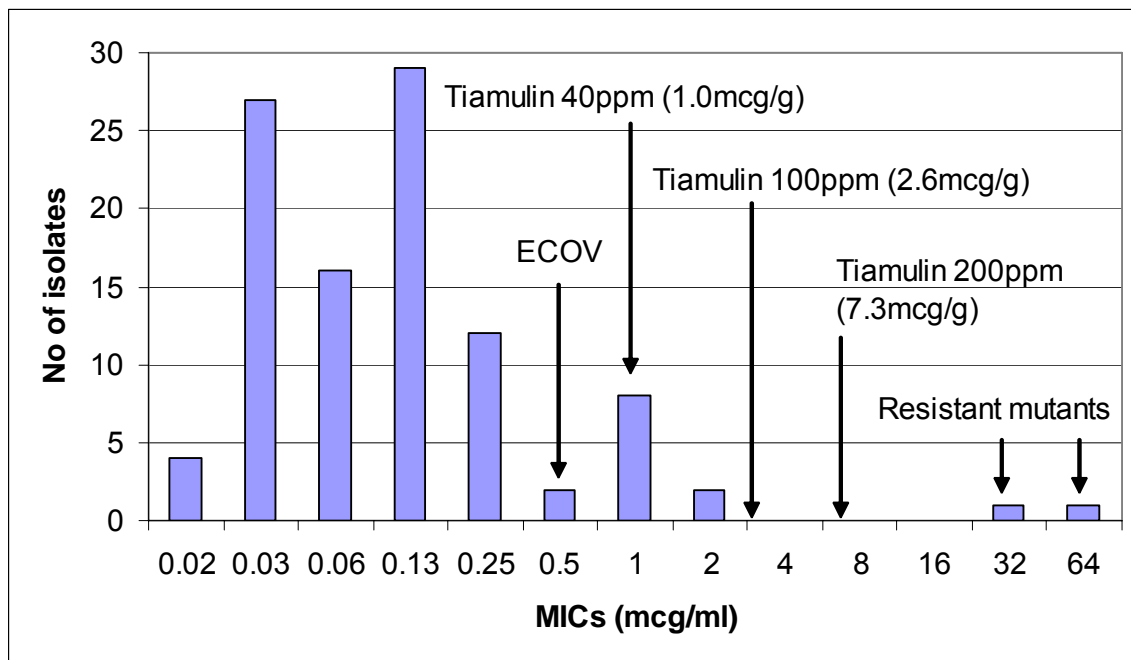


Fig.7. Tiamulin concentrations in caecal contents in relation to MIC susceptibility pattern against *B. hyodysenteriae*.

The ability to kill susceptible bacteria and first-step mutants may explain why tiamulin has maintained much of its activity against *B. hyodysenteriae* over the last 30 years.

Where antimicrobial concentrations cannot be kept above the MPC, another approach in human medicine has been to use combinations of drugs, which both act on the organism but in different ways. It is considered unlikely that the organism being treated will develop resistance to two different antibiotics at the same time. The use of penicillins and aminoglycosides has been commonly used e.g. penicillin and streptomycin in veterinary medicine to piperacillin and gentamicin for *Pseudomonad* infections in man and more recently meropenem and tobramycin (Tam et al, 2005).

#### d. Therapeutic approach and mutant risk development



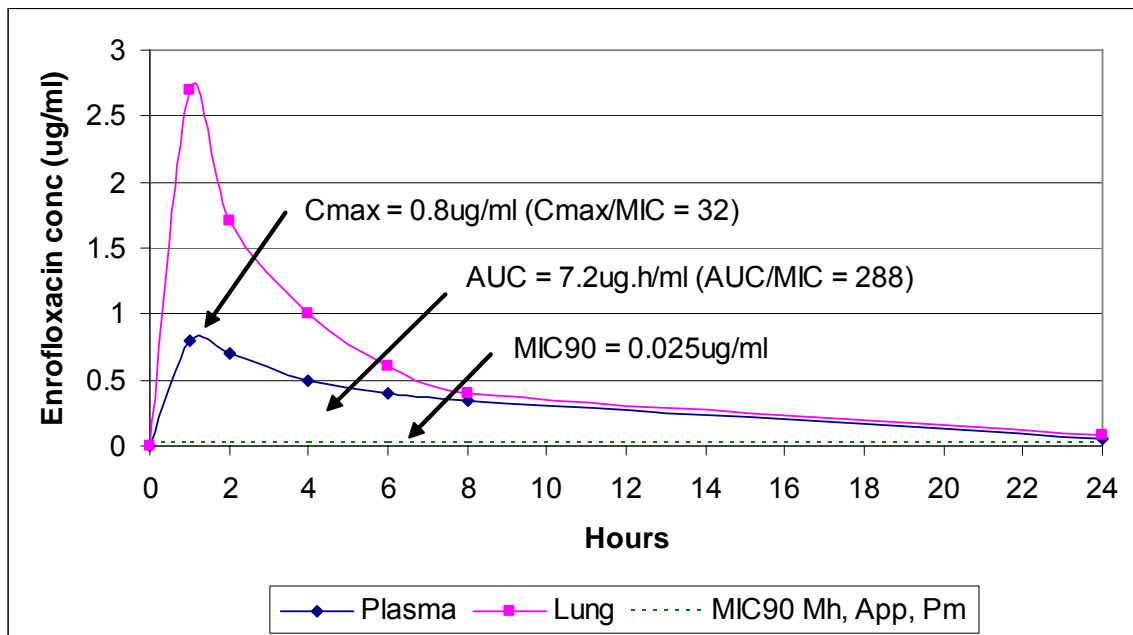
These concepts can be used to compare therapeutic approaches and a risk to likely mutant development ascribed (Table 1).

**Table 1.** Therapeutic approach and comparison of mutant risk

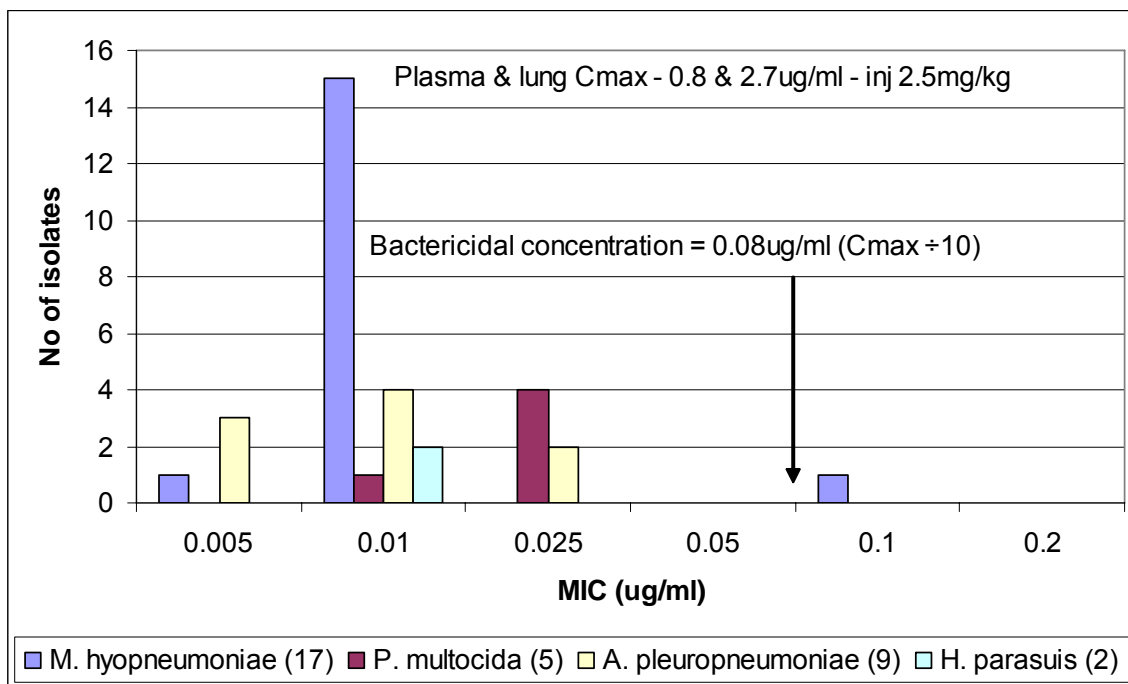
Approach	Bacterial numbers	Mutant selection window	Mutation risk
Prevention levels (low dose)	Low $<10^6$	In (often prolonged use)	Moderate
Metaphylaxis (high dose)	Low $<10^6$	In Above	Low Very low
Treatment (high dose)	High $>10^6$	In Above	High Moderate
Combination	High $>10^6$	In Above	Low (double mutation) Very low

### e. Incongruities with PK/PD integration

In some cases, it has been difficult to use PK/PD integration to assess the efficacy of some antimicrobial drugs, particularly against respiratory bacteria. When enrofloxacin was first introduced the MICs for the majority of isolates of *M. hyopneumoniae*, *P. multocida*, *A. pleuropneumoniae* and *H. parasuis* were  $\leq 0.025\mu\text{g/ml}$  (Hannan et al, 1989). The PK in plasma (Scheer, 1987) and PD integration fits the criteria described previously (Fig. 8 & 9) and although enrofloxacin does concentrate in lung tissue to a certain degree, plasma concentration would appear to be the most critical parameter for the PK aspects.



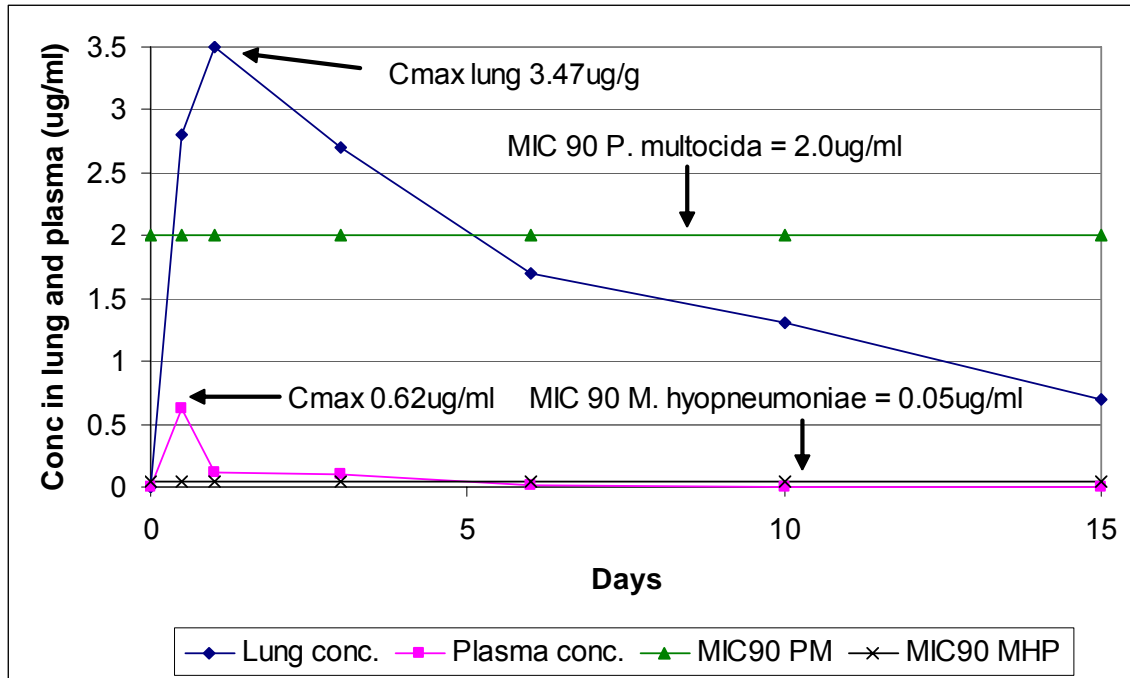
**Fig. 8.** PK curves in plasma and lung for enrofloxacin and PK/PD integration for respiratory organisms



**Fig. 9.** Enrofloxacin Cmax relationship to MICs for respiratory pathogens

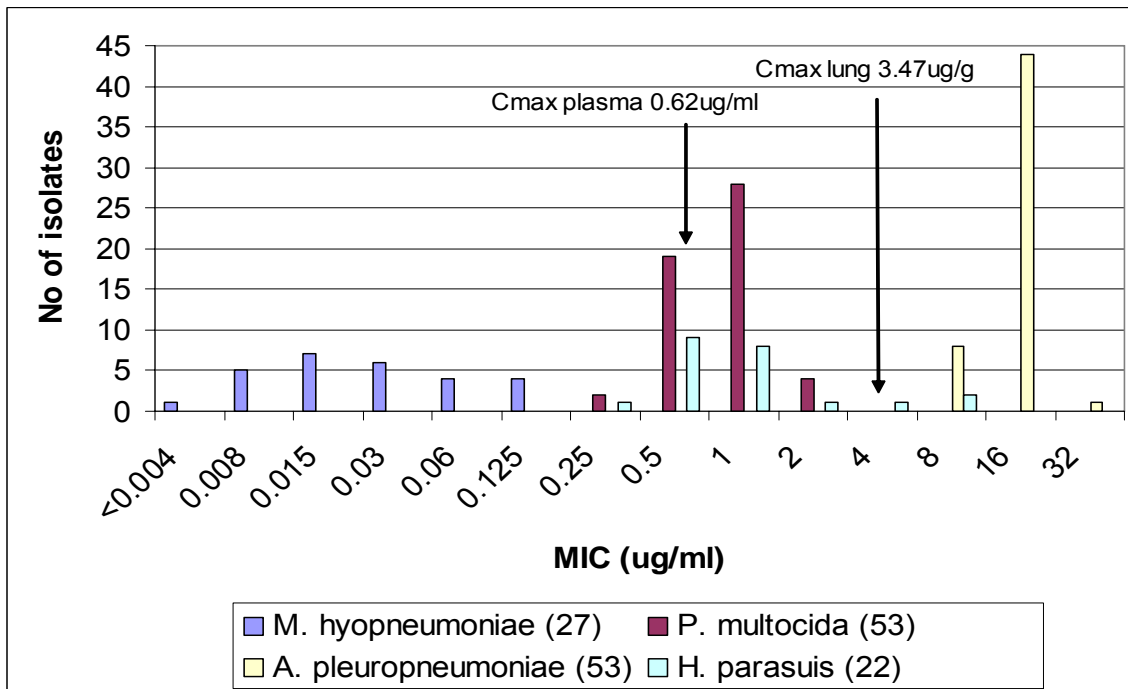
The majority of respiratory pathogens should be effectively killed by and injection of 2.5mg/kg bodyweight as the Cmax / 10 is 0.08µg/ml, well above the MIC of 0.025µg/ml.

With tulathromycin a different picture is displayed. Benchaoui et al, 2004 described the PK of tulathromycin in pigs following a 2.5mg/kg injection. The PK profile is markedly different from enrofloxacin with regard to duration of activity (days) and comparative concentration in the plasma and lung tissue (Fig. 10).



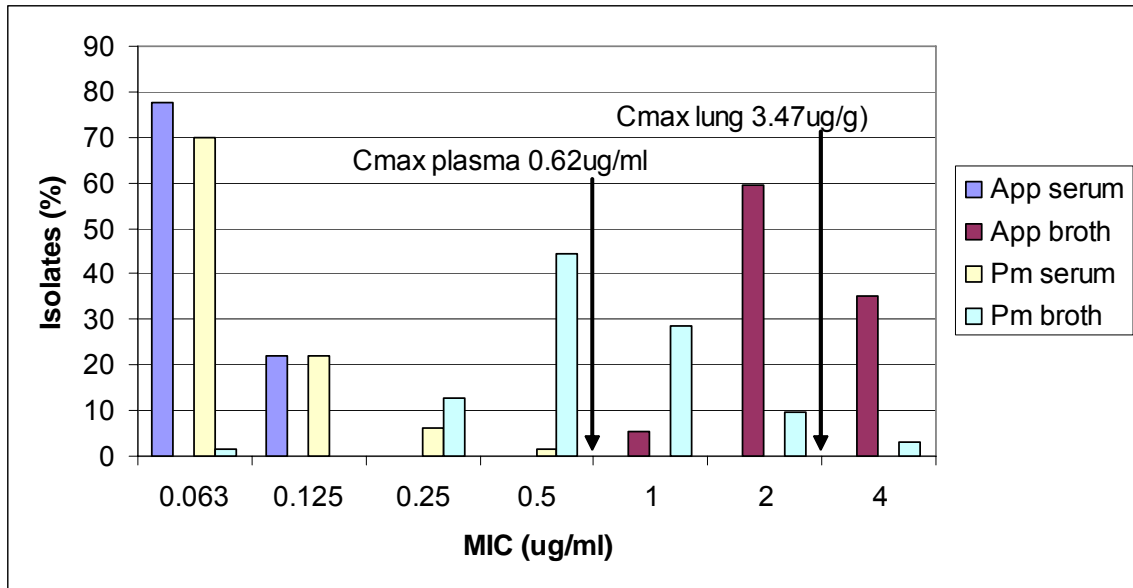
**Fig 10.** PK curves in plasma and lung for tulathromycin and MIC 90 for *M. hyopneumoniae* and *P. multocida*

There is a marked difference in MIC 90s between *M. hyopneumoniae* and *P. multocida*. When other respiratory bacteria were examined there were even larger differences, especially for *A. pleuropneumoniae* (Godinho, 2008; Godinho et al, 2005), against which the drug is active in vivo (Fig. 11).



**Fig. 11.** Comparison of MICs for tulathromycin against various respiratory pathogens

When the bacteria were tested using the CLSI standard tests for respiratory organisms, very high MIC levels were obtained. Under these circumstances, it is very tempting to look at other PK parameters, such as lung concentrations and macrophage concentrations for these antibiotics. They have been described for tilmicosin (Stoker et al, 1996) and also tiamulin (Burch & Klein, 2008) to explain PK/PD integration. However, when non-CSLI methods are used which incorporates serum into the culture medium much lower, probably more representative MICs are achieved (Fig. 12).



**Fig. 12.** Comparison of MICs for tulathromycin against *A. pleuropneumoniae* and *P. multocida* when grown in CSLI broth or in media containing serum

The susceptibility of the bacteria to tulathromycin increased by 3-5 dilutions, and brought the MICs of the organisms into the plasma PK range like most other antibiotics.

## CONCLUSIONS

The use of PK/PD integration can be very helpful in selecting the best medicines and to employ them correctly to get the best programme. It is a very powerful tool for the interpretation of data because if the data does not fit, then usually some aspect is incorrect, e.g. the MIC method is wrong, or possibly the target tissue concentration is incorrect, possibly due to high protein binding etc. The PK/PD relationships devised from the treatment of man are very useful for treating animals, although some extra aspects need to be included. Veterinary medicine often uses older drugs such as the tetracyclines, which are primarily bacteriostatic. Increasingly, the relationship of PK to antimicrobial resistance patterns is also helpful to determine the effects of past exposure and resistance development. The new concepts of epidemiological breakpoints, mutation patterns and clinical breakpoints are also useful to the clinician to improve dosing schedules and

where possible to exceed the mutant prevention concentration, so that the chances of mutant selection and resistance development are reduced.

In veterinary medicine there are also other constraints to consider, such as economic cost of medication, potential toxicity by increasing doses or reducing palatability in the case of oral administration and also the effects on withdrawal periods. However, there are opportunities with PK/ PD integration techniques to improve our understanding of the workings of antibiotics to help veterinarians use them prudently and more effectively to reduce resistance development in both animals and man and to maintain their efficacy and availability for the future.

## REFERENCES

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, pp115-118

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

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

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 11<sup>th</sup> International Pig Veterinary Society Congress, Lausanne, Switzerland, p. 43.

Drlica, K. (2003) The mutant selection window and antimicrobial resistance. Journal of Antimicrobial Chemotherapy, 52, 11-17

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.

Godinho, K.S. (2008) Susceptibility testing of tulathromycin: Interpretative breakpoints and susceptibility of field isolates. Veterinary Microbiology, 129, 426-432.

- 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.
- Lees, P. and Aliabadi, F.S. (2002) Rational dosing of antimicrobial drugs: animals versus humans. *International Journal of Antimicrobial Agents*, 19, 269-284
- Lees, P., Svendsen, O. and Wiuff, C. (2008) Chapter 6: Strategies to minimise the impact of antimicrobial treatment on the selection of resistant bacteria. In *Guide to Antimicrobial Use in Animals*. Eds, Guardabassi, L., Jensen, L.B. and Kruse, H., Blackwell Publishing, Oxford, UK pp. 77-101
- 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.
- Schentag, J.J. (2000) Clinical pharmacology of the fluoroquinolones: studies in human dynamic/kinetic models. *Clinical Infectious Disease*, 31 (Supplement 2) S40-44
- 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 14<sup>th</sup> International Pig Veterinary Society Congress*, Bologna, Italy, p. 656.
- Tam, V.H., Schilling, A.N., Neshat, S., Poole, K., Melnick, D.A. and Coyle, E.A. (2005) Optimization of Meropenem minimum concentration/MIC ratio to suppress in vitro resistance of *Pseudomonas aeruginosa*. *Antimicrobial Agents and chemotherapy*, 49, 12, 4920-4927
- Wiuff, C., Lykkesfeldt, J., Aarestrup, F.M. and Svendsen, O. (2002) Distribution of enrofloxacin in intestinal tissue and contents of healthy pigs after oral and intramuscular administrations. *Journal of Veterinary Pharmacology and Therapy*, 25, 335-342
- Veterinary Medicines Directorate (2008) Report: Sales of antimicrobial products for use as veterinary medicines, antiprotozoals, antifungals, growth promoters and coccidiostats in the UK in 2007.