Pharmacokinetics / Pharmacodynamics of Denagard® and Econor® and Their Use for Enteric and Respiratory Disease Control in Pigs

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Introduction

The study of the pharmacokinetics (PK) of antibiotics, their relationship to pharmacodynamic (PD) activity against bacteria and clinical effect has made an important difference to our understanding of how these drugs work. In addition, they can be integrated to see how susceptibility patterns have come to develop with time and can also give a useful key as to future control of antibiotic resistance development.

Each drug has its own PK profile and PD activity and it is the purpose of this paper to review the activity of tiamulin hydrogen fumarate (Denagard® - Novartis) and valnemulin (Econor® - Novartis) against *Brachyspira hyodysenteriae, Lawsonia intracellularis* and *Mycoplasma hyopneumoniae* and the treatment and control of these common infections in pigs.

Basic PK/PD and clinical relationships

Pharmacokinetics

From a PK perspective, the most commonly used measurement is the concentration of the unbound drug in plasma or serum, for systemic and respiratory infections. In enteric infections, the concentration of the drug in the contents of the intestine at specific sites is also highly useful. In some cases the data is not available but can be extrapolated using gut flow models (Burch, 2005). Binding of drug to gut contents can also have an impact on local bioavailability, but there is also very little data available.

The route of administration is also important. Injectables and bolus doses usually give higher peaks of plasma concentration (Cmax), whereas when a drug is given in water or feed during the day the level is usually lower and flatter. The area under the curve (AUC) may be the same if the bioavailability is the same but again this might be influenced by the absorption, metabolism in liver and rate of excretion of a drug (see Figure 1).

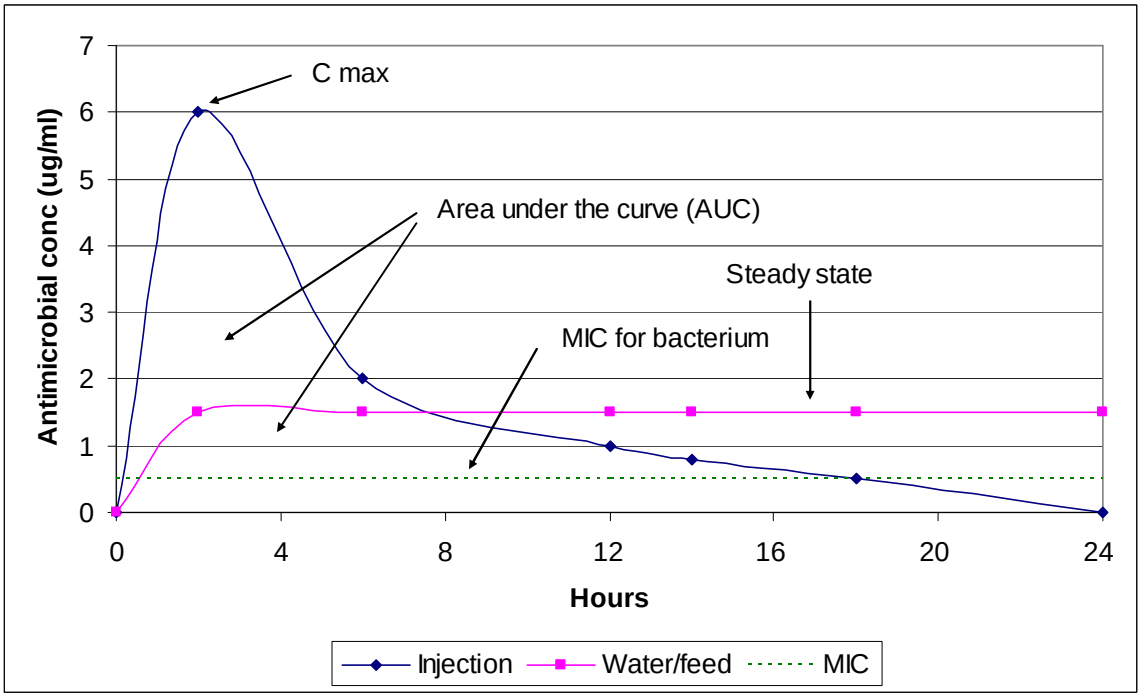
Pharmacodynamics

A commonly used measurement is the minimum inhibitory concentration (MIC) of the drug against a certain organism. When 10 or more strains of a bacterium are tested the MIC 50 and MIC 90 may be calculated to give an idea of susceptibility to that drug. A susceptibility or resistance pattern can also be drawn and the determination of ‘wild types’ populations, bacteria that have not been exposed to antibiotics, mutants and resistant organisms can also be demonstrated.

The way the drug acts on a bacterium is also important. The MIC is the inhibitory concentration, i.e. stops it growing but the minimum bactericidal concentration (MBC) is the concentration that kills the bacterium. The MBC/MIC ratio can be 1: 1 for some drugs like the fluoroquinolones and the aminoglycosides, which act primarily in a bactericidal

way, but others, including tiamulin and valnemulin, are primarily bacteriostatic in action and it is not until higher concentrations are achieved that a bactericidal effect occurs and the MBC/MIC ratio can be as low as 2:1 but can be much higher at 40: 1, which is sometimes the case for the tetracyclines. It is drug and organism dependent.

Figure 1. Typical PK/PD relationships following an injectable or oral administration of a drug



When a drug acts primarily in an inhibitory way it is also dependent on an active immune system to eliminate the infection. With some disease in pigs, such as porcine reproductive and respiratory virus (PRRSV) and porcine circovirus type 2 (PCV2) the immune system is damaged and the pig does not always respond clinically to treatment and the infectious agent may not be eliminated and can continue to cause disease.

Basic PK/PD relationships

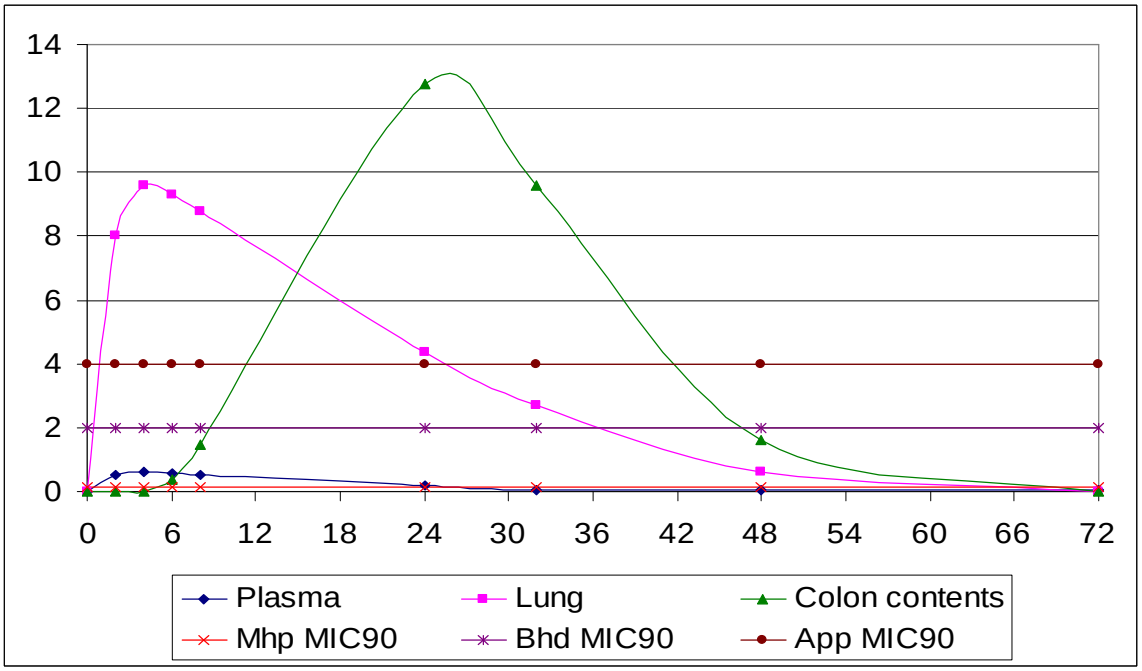
For bactericidal compounds, the Cmax/MIC ratio of about 10-12: 1 is usually highly effective in killing bacteria. Fluoroquinolones and aminoglycosides are commonly considered to be concentration dependent in their killing activity. The AUC 24h is also a very useful measurement for estimating the likely effect of the drug on the bug and can be applied to most antibiotics. For bactericidal drugs the AUC 24h/MIC ratio is approximately 100-120: 1 or effectively 4-5 times the MIC over a 24 hour period. Some antibiotics such as the penicillins act in a time dependent way, so the Time greater than the MIC (T >MIC) is a useful measurement for them. For bacteriostatic compounds such as tiamulin and valnemulin the AUC 24h/MIC can be much higher than 100-120: 1 to achieve a killing effect but in some cases such as *B. hyodysenteriae* the MBC/MIC is about 2: 1 so a good killing effect can be achieved at 4-8 times the MIC. The pleuromutilins are both time and concentration dependent in their killing activity. The

steady state (SS) is also a useful measurement especially after water or feed medication because of the flatness of the curve, and is also useful in calculations for lower gut concentrations from the ileum down, as again they are relatively flat and stable.

Tiamulin pharmacokinetics

Tiamulin injection pharmacokinetics
Tiamulin when injected displays a whole range of PK concentrations in various tissues (McKellar et al, 2004) (see Figure 2).

Figure 2. Tiamulin injection pharmacokinetics



Tiamulin achieves relatively low levels in plasma in comparison with concentrations in the lung (Cmax 15.7 times higher; AUC lung/ AUC plasma 18.1times higher) and in the colon contents (Cmax 21 times higher). The drug is excreted via the bile into the gut contents and concentrates in the colon. This gives tiamulin its special qualities of treating mycoplasma infections, ileitis, colitis and swine dysentery.

Tiamulin and valnemulin oral pharmacokinetics
When given orally, both tiamulin and valnemulin are absorbed to a high degree and metabolised in the liver. The plasma levels are quite low because of this but they concentrate in lung tissue as they are very lipid soluble and have a good distribution. They are excreted in the bile also and join the gut contents, with whatever was not absorbed originally, and they pass through the ileum, bathing the ileal wall, where *L. intracellularis* colonises and then concentrate in the colon. The ileal concentration is approximately 25-30% of the colon contents concentration, and in our modelling we have used a figure of 29% (Burch, 2005).

The concentration of tiamulin in lung and colon contents following oral administration of tiamulin via the drinking water and via feed was measured by Anderson et al. (1994).

Table 1. Tiamulin oral pharmacokinetics (Anderson et al, 1994) plus Japanese usage levels estimated concentrations

Tiamulin dose rate **In water**	**13.2mg/kg bwt** *120ppm tiamulin*	**20.9mg/kg bwt** *180ppm tiamulin*
Tiamulin lung conc. (µg/g)	4.3	8.5
Tiamulin plasma conc. E (µg/ml)	0.24	0.47
Tiamulin colon contents (µg/g)	5.59	18.58
In feed	*220ppm*	*50-300ppm (Japan) E*
Tiamulin lung conc. (µg/g)	1.99	0.24-2.7
Tiamulin plasma conc. E (µg/ml)	0.11	0.025-0.15
Tiamulin colon contents (µg/g)	8.1	1.8-11
Tiamulin ileal contents E (µg/g)	2.4	0.53-3.2

Key: E = Estimated

The concentration for valnemulin in lung and colon contents was also reported (Novartis Report, 1998).

Table 2. Valnemulin concentrations in lung and colon contents following in-feed medication

Valnemulin	30ppm (J)	75ppm	100ppm (J)	200ppm
Lung concentration (µg/g)	-	0.04	0.12	0.23
Colon contents (µg/g)	0.64	1.6	2.6	5.2
Ileal contents (µg/g) E	0.19	0.46	0.75	1.5

Key: E = Estimated; J = Japanese usage levels

Valnemulin also concentrates in the lung and has low plasma levels, but the MICs against *M. hyopneumoniae* are also extremely low. It accumulates in colon and ileal contents, similarly to tiamulin and is highly active against *B. hyodysenteriae* and *L. intracellularis*.

Treatment and prevention of ileitis

Porcine proliferative enteropathy (‘ileitis’) is caused by *L. intracellularis*. The organism appears to prefer the area of the ileum, caecum and proximal colon to colonise and multiply and once ingested, rapidly penetrates the cells lining the gut. Although MIC tests have been carried out both intracellularly and extracellularly, the intracellular MIC is more representative, when it comes to determining a PK/PD relationship, as the drug bathes the cells lining the gut as it passes by and the organism is usually intracellular at that stage.

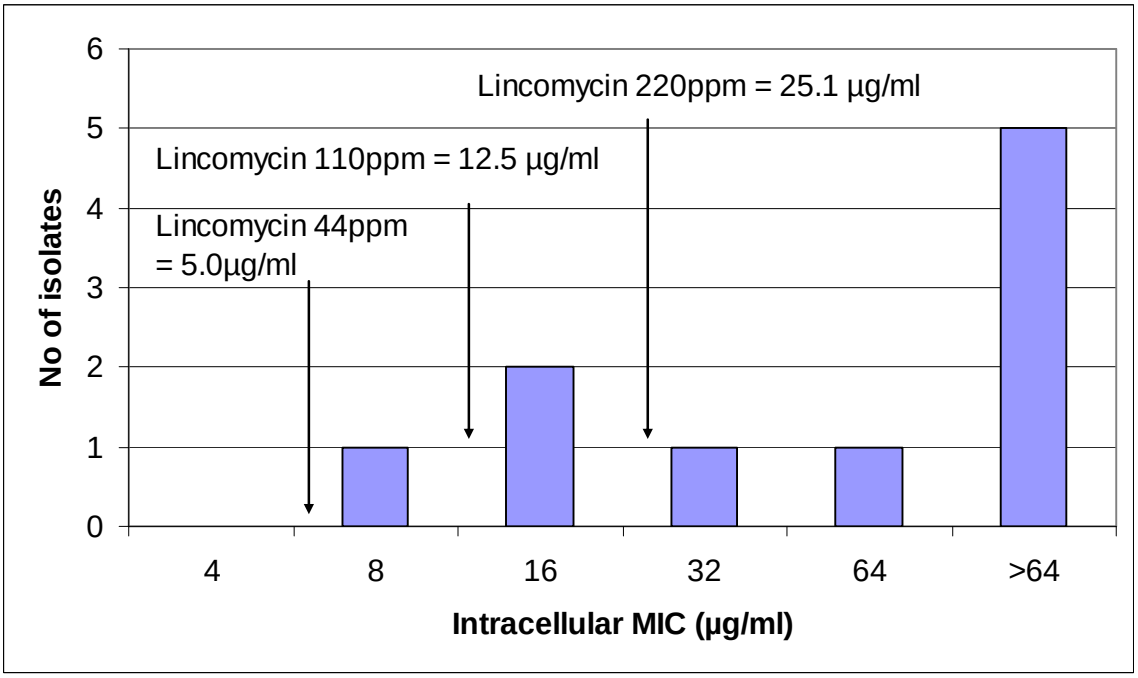
Recent work by Wattanaphansak et al (2009) reported on the intracellular MICs for both valnemulin and tiamulin against 6 US and 4 EU isolates.

Table 3. Intracellular MICs against *L. intracellularis*

Antimicrobial	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Tiamulin	≤0.125	≤0.125	≤0.125
Valnemulin	≤0.125	≤0.125	≤0.125
Chlortetracycline	8.0	64	0.125-64
Lincomycin	32	>128	8.0->128
Tylosin	2.0	8.0	0.5-8.0
Carbadox	≤0.125	≤0.125	≤0.125-0.25

Tiamulin and valnemulin had consistently low MICs, whereas lincomycin (see Figure 3) and chlortetracycline had some very high MICs suggesting resistance development.

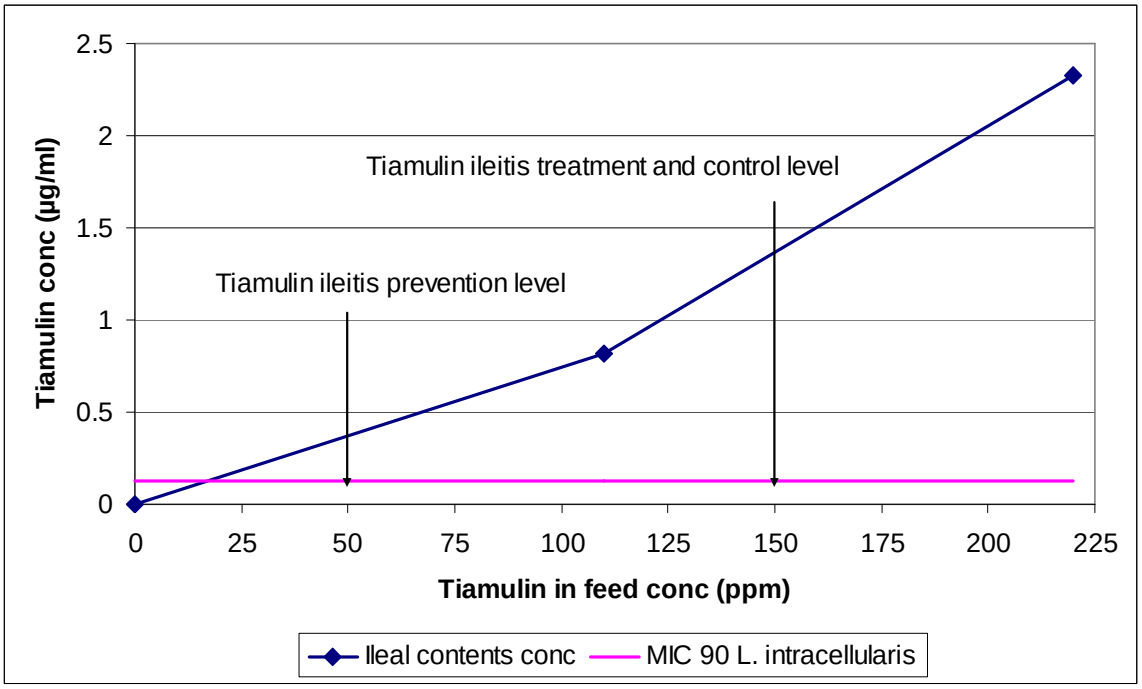
Figure 3. Lincomycin MICs against *L. intracellularis* and estimated ileal contents concentrations (DeGeeter et al, 1980)



Tiamulin

The PK/PD relationships are demonstrated in Figure 4.

Figure 4.PK/PD relationships of tiamulin against *L. intracellularis*



These results suggest there should be good inhibition of *L. intracellularis* at 50ppm tiamulin and a very strong treatment effect at 150ppm with ileal content concentration at 3 and 11 times the MIC.

This was confirmed in an artificial infection study (McOrist et al, 1996) when pigs were artificially challenged and given a prevention dose of 50ppm tiamulin for 21 days from infection and 150ppm tiamulin for 14 days, 7 days after infection. No gross or histopathological lesions were detected in the treated groups. The MIC of the organism used was ≤0.125µg/ml (Wattanaphansak et al, 2009).

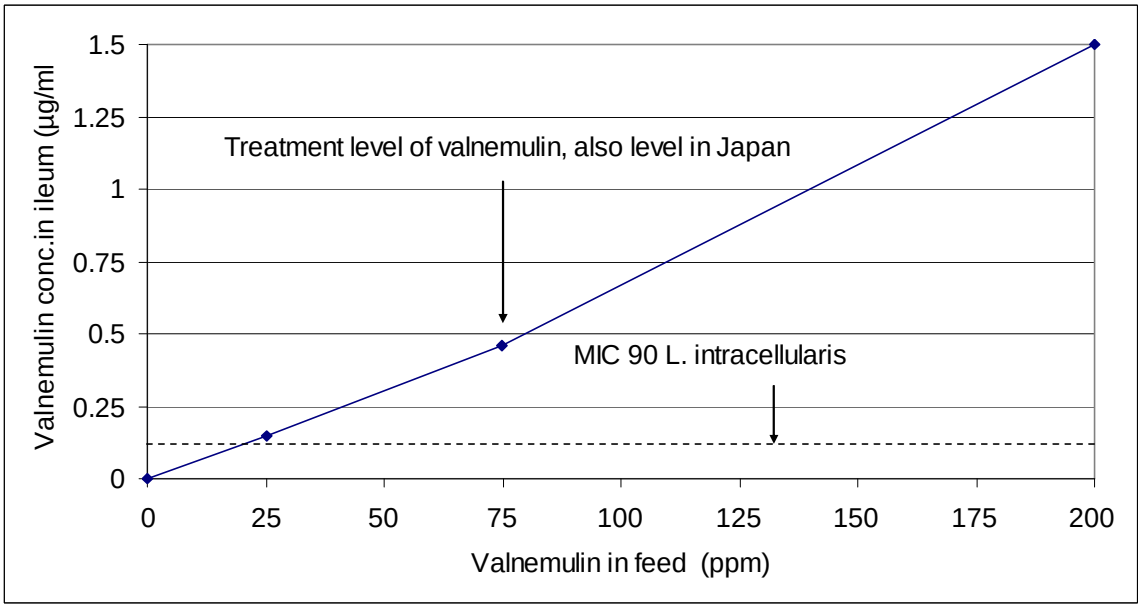
Table 4. Treatment and prevention of ileitis with tiamulin in an artificial challenge study

Treatment group	Gross lesions pigs affected (%)	Histological lesion score ileum (%)	Histological lesion score caecum (%)
Infected untreated control	86	42	46
Prevention: Tiamulin 50ppm from -2 to 21days	0	0	0
Treatment: Tiamulin 150ppm 7-21 days	0	0	0

Valnemulin

The PK/PD relationship is demonstrated in Figure 5.

Figure 5. PK/PD relationships of valnemulin against *L. intracellularis*



At approximately 25ppm valnemulin only an inhibitory effect can be expected, but at 75ppm and above a bactericidal effect could be anticipated.

In the artificial infection study with valnemulin (McOrist et al, 1998) pigs were challenged with a *L. intracellularis* infected cell culture and received valnemulin at 0, 25, 37.5 and 50ppm for prevention during the 21 day trial and 75ppm for treatment from day 7 for 14 days. There was a dose-related response for prevention with 50ppm giving the best results, eliminating gross lesions although histological lesions could be detected still. At 75ppm valnemulin for treatment, there were no gross lesions not histological lesions recorded.

Table 5. Treatment and prevention of ileitis with valnemulin in an artificial challenge study

Valnemulin conc. in feed (ppm)	Pigs with lesions (%)	Histological lesion score (%)
0	71	100
25 (Prevent)	29	46
37.5 (Prevent)	20	22
50 (Prevent)	0	9
75 (Treat 7-21 days)	0	0

Treatment of swine dysentery

In a recent study, the MICs of a number of antibiotics were recorded against 77 Japanese isolates of *B. hyodysenteriae* (Adachi et al, 2008).

Table 6. MICs of various antibiotics against 77 Japanese *B. hyodysenteriae* isolates

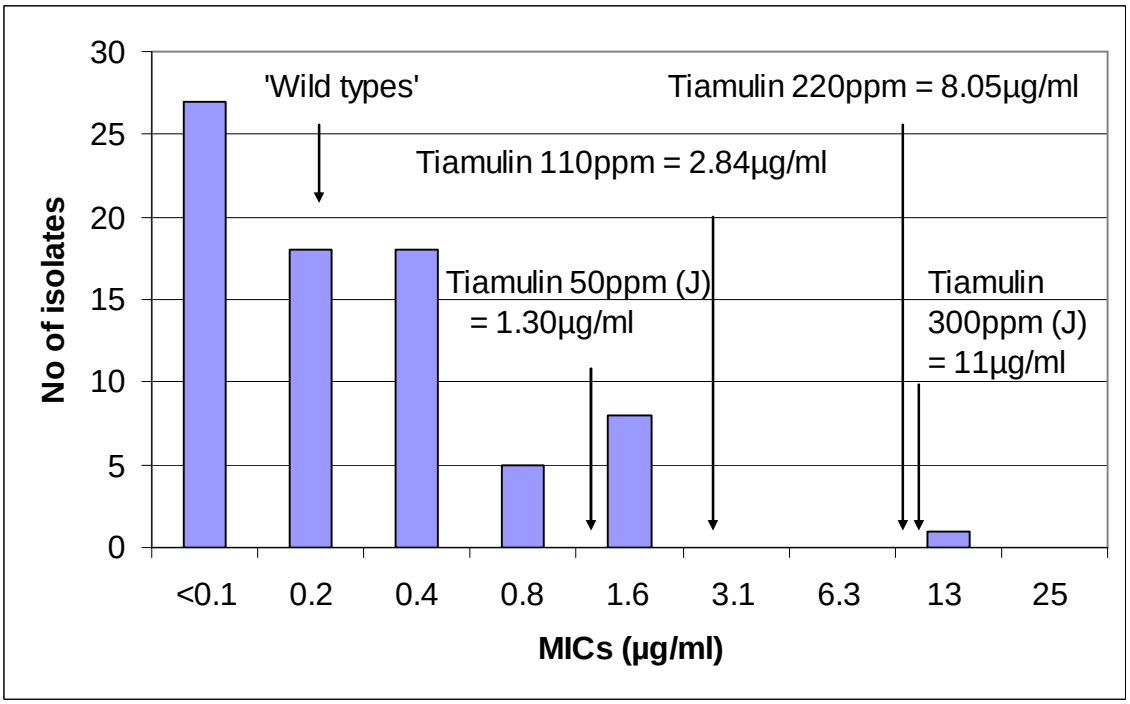
Antibiotic	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Tiamulin	0.2	1.6	<0.1-13
Valnemulin	<0.1	<0.1	<0.1-1.6
Tylosin	100	>100	<0.1->100
Tylvalosin	13	100	<0.1->100
Lincomycin	3.1	>100	<0.1->100

Tiamulin and valnemulin still show a very high level of activity against *B. hyodysenteriae*, whereas tylosin displays a major resistance and tylvalosin, a tylosin derivative, and lincomycin also show some marked resistance. With this number of isolates susceptibility patterns can be demonstrated and compared with the antibiotic concentrations achieved in the colon contents at the various usage levels.

Tiamulin

Figure 6 shows quite distinctly the 'wild type' pattern for tiamulin against *B. hyodysenteriae* up to 0.8µg/ml. There is a small peak at 1.6µg/ml suggesting a first-step mutant and at 13µg/ml there is a resistant, second-step mutant, which has an MIC above usual therapeutic levels of tiamulin.

Figure 6. MIC susceptibility patterns correlated with tiamulin colon contents concentrations



Key: J = Japanese usage level

In an artificial infection study (Taylor 1982) tiamulin was used for treatment at 0, 50, 80, 120 and 180ppm for 14 days. The MIC of the isolate used was 0.5µg/ml. A definite dose

response could be seen with a bactericidal effect observed at approximately 6 times the MIC with 120ppm and above.

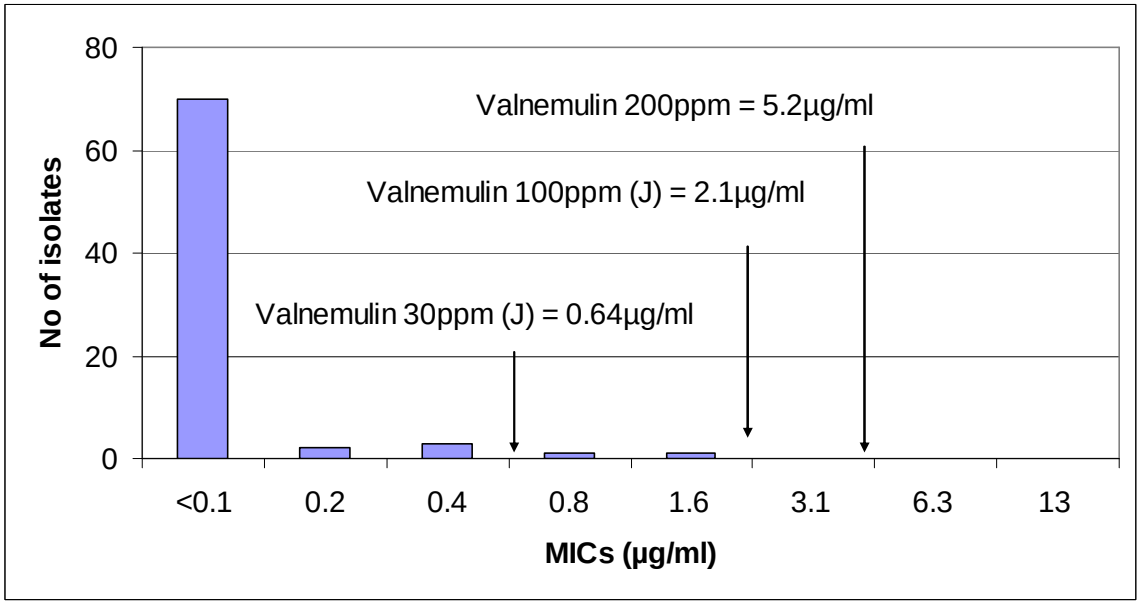
Table 7. Treatment of swine dysentery with tiamulin in an artificial challenge study

Tiamulin in feed (ppm) days 0-14	Swine dysentery (clinical & bacteriological) day 0	Re-isolation of *B. hyodysenteriae* day 14
0	3/5	3/5
50	2/5	1/5
80	2/5	1/5
120	4/5	0/5
160	3/5	0/5

Valnemulin

The Japanese strains of *B. hyodysenteriae* showed a very high level of susceptibility to valnemulin (see Figure 7). There is a pattern of mutation at about 0.2µg/ml but very few organisms seemed to be involved.

Figure 7. MIC susceptibility patterns correlated with valnemulin colon contents concentrations



Key: J = Japanese usage levels

In a dose-titration study where pigs were challenged with a *B. hyodysenteriae* isolate with an MIC of 0.025µg/ml, and treated for 10 days (Burrows et al, 1996) levels of 75ppm and above eliminated lesions but 100ppm eliminated the infection as well (see Table 8).

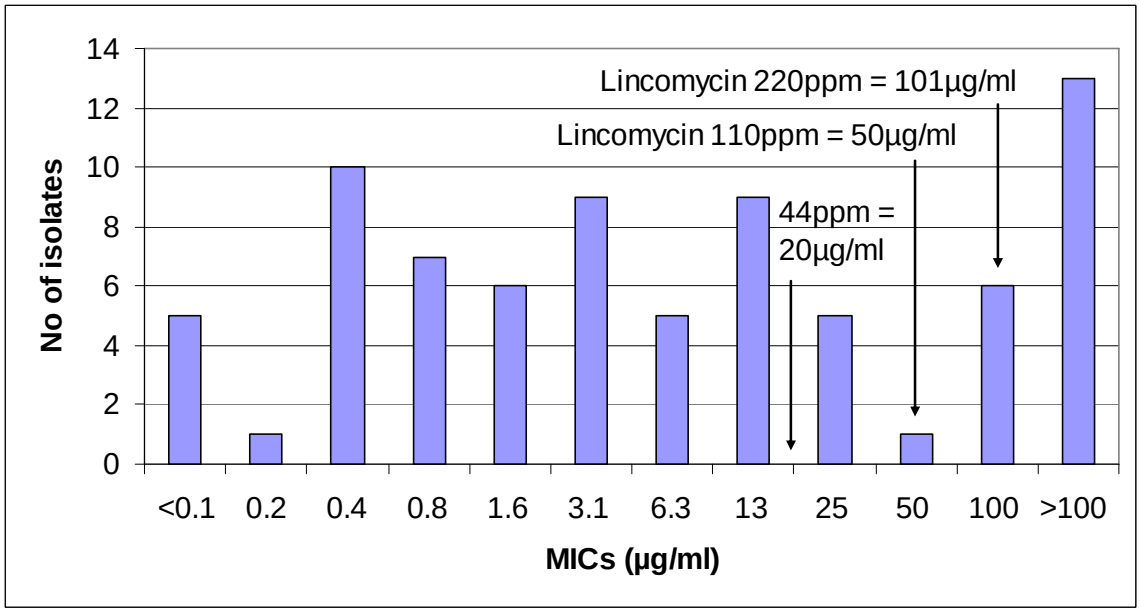
Table 8. Treatment of swine dysentery with valnemulin in an artificial challenge study

Valnemulin inclusion rate (ppm) in feed for 10 days	Swine dysentery lesions at post mortem	Re-isolation of *B. hyodysenteriae*
0	8/8	8/8
50	2/8	5/8
75	0/8	1/8
100	0/8	0/8

Other antibiotics

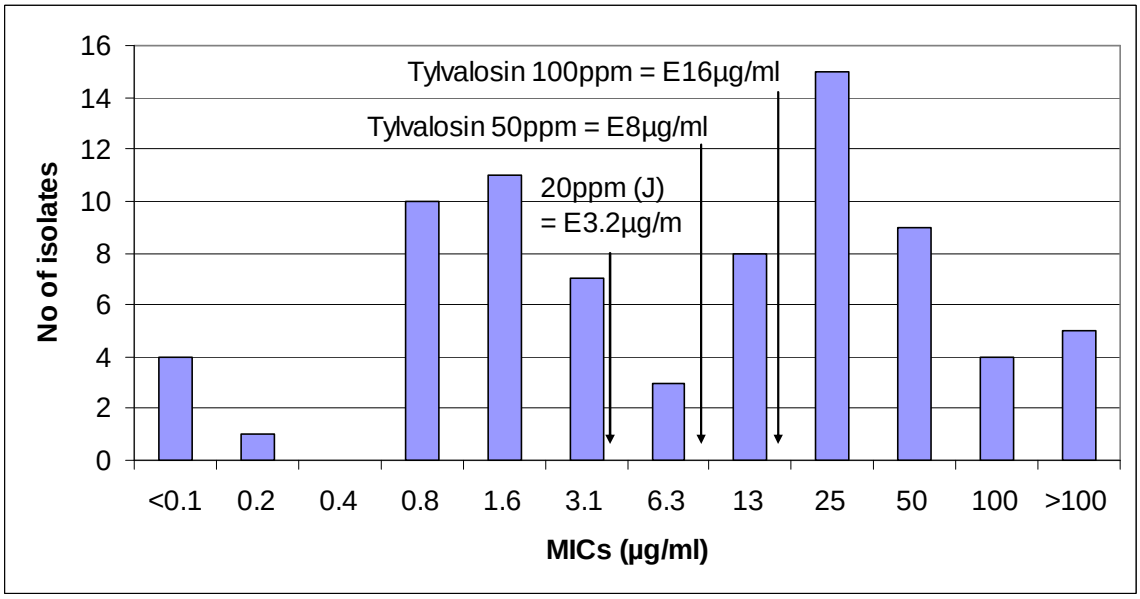
By comparison, lincomycin has a different susceptibility pattern, which correlates well with its colonic contents concentration (DeGeeter et al, 1980) and there is a definite resistance pattern of 17-25%, depending on the inclusion rate used (see Figure 8).

Figure 8. MIC susceptibility patterns correlated with lincomycin colon contents concentrations



Tylvalosin shows a more marked, biphasic susceptibility pattern with a resistance of 43% (see Figure 9).

Figure 9. MIC susceptibility patterns correlated with tylvalosin colon contents concentrations



Key: J = Japanese usage levels; E = estimated

Enzootic pneumonia

Enzootic pneumonia is caused by *M. hyopneumoniae* and is a chronic respiratory infection in pigs, taking several weeks for the lesions to develop and resolve. Mixed with other respiratory viruses such as PRRSV and PCV2, as well as bacteria such as *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*, it causes major respiratory problems associated with the porcine respiratory disease complex (PRDC).

Recent work in Japan (Kobayashi et al, 2008) looked at the MICs of several antimicrobials against 90 isolates of *M. hyopneumoniae*.

Table 9. MICs of various antimicrobials against 90 *M. hyopneumoniae* isolates, compared with levels found in plasma after in-feed administration

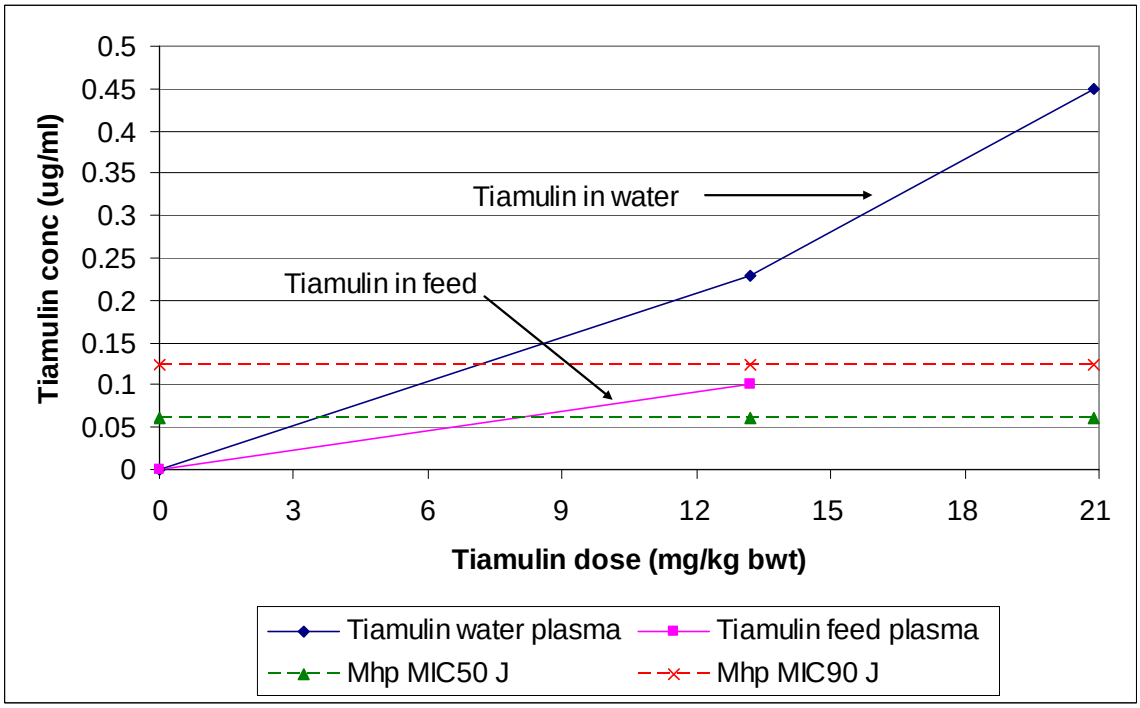
Antimicrobial	Conc in feed (ppm)	Conc in plasma (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Tiamulin	220	0.11E	0.06	0.125	0.03-0.125
Valnemulin	200	<0.05	0.002	0.004	0.002-0.004
Oxytetracycline	400	0.17	1.0	2.0	0.25-4.0
Lincomycin	220	0.16	0.25	0.5	0.125->64
Tilmicosin	400	0.2	0.25	0.5	≤0.25->16
Tylosin	110	0.03E	0.06	0.25	0.06->64
Enrofloxacin	150	0.24	0.125	0.125	0.06-1.0

All of the antibiotics are primarily bacteriostatic, only enrofloxacin is bactericidal. Plasma concentrations are surprisingly low for most of the antibiotics given via the in-feed route and have primarily an inhibitory effect.

Tiamulin

Anderson et al (1994) demonstrated that there was a major difference in lung concentrations when tiamulin was given in feed and via the drinking water. Dose for dose, water gave higher lung concentrations and these can be extrapolated to plasma levels by dividing by 18.1 (see Figure 10).

Figure 10. Tiamulin plasma kinetics administered via different oral routes and their association with *M. hyopneumoniae* MICs



In feed, tiamulin will reach inhibitory levels for all Japanese isolates, especially if it is projected to the 300ppm inclusion level (approximately 15mg/kg bodyweight).

In parts of Europe and the US, tiamulin is authorised for use at levels in the drinking water up to 180ppm and at these levels there is a major effect against *M. hyopneumoniae*, but also *A. pleuropneumoniae*.

Two separate artificial infection studies looked at the use of tiamulin for the treatment of *M. hyopneumoniae*.

Trial 1 – (Underdahl and Szanto, 1976). Gnotobiotic piglets were treated 7 days after infection for 5 days using tiamulin at 4.4 and 8.8 mg/kg bodyweight administered twice daily via the milk. Pigs were necropsied 21 days post infection and the MIC of the organism was 0.1µg/ml.

Trial 2 – (Hannan et al, 1982). Pigs were treated at 10mg/kg twice daily 14 days after they had been infected with lung homogenate and necropsied 38 days after infection. The trial was repeated twice and the MICs of the strains re-isolated were 0.1-0.25µg/ml.

Table 10. Combined trial results – lung lesion scores (%)

Trial	Tiamulin 0 mg/kg	Tiamulin 8.8 mg/kg	Tiamulin 17.6 mg/kg	Tiamulin 20 mg/kg
1	100	100	21 (-79%)	-
2a	100	-	-	22 (-78%)
2b	100	-	-	2 (-98%)

At these dose rates there was a substantial reduction in lesions.

Valnemulin

Has shown unprecedented activity against *M. hyopneumoniae* in vitro and in vivo also showed exceptional efficacy. In two artificial challenge studies (Morgan et al, 1996) using lung homogenate containing *M. hyopneumoniae*, valnemulin was given for 21 days in feed at a variety of inclusion rates following infection with two isolates with different MICs.

Table 11. Efficacy of valnemulin in feed against *M. hyopneumoniae*

Valnemulin concentration in feed (ppm)	0	100	200	400
Trial 1 (MIC 0.016µg/ml)	100	100	54 (-46%)	29 (-71%)
Trial 2 (MIC 0.008µg/ml)	100	-	21 (-79%	-

Conclusions

Tiamulin and valnemulin have very special pharmacokinetics and the antibiotics are well absorbed from the gut and go via the plasma to concentrate in lung tissue. Excretion is via the bile so bioactive residues and parent compound are also re-excreted into the gut and concentrate in the ileal and colonic contents.

Plasma concentrations are normally associated with efficacy against *M. hyopneumoniae* and administration via the water gives higher lung and plasma levels of tiamulin than via the feed. Administration via the feed is thought to slow the absorption of tiamulin and reduce its bioavailability but higher concentrations are achieved in the gut when given in feed.

Both tiamulin and valnemulin are very active and effective against *L. intracellularis* the cause of ileitis and also *B. hyodysenteriae* the cause of swine dysentery. They are also very active against *B. pilosicoli* the cause of colitis in pigs.

Japanese isolates of *B. hyodysenteriae* and *M. hyopneumoniae* appear very susceptible to tiamulin and valnemulin in comparison with some of the older antibiotics, which are commonly used.

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