PHARMACOKINETIC, PHARMACODYNAMIC AND CLINICAL CORRELATIONS RELATING TO THE THERAPY OF *LAWSONIA INTRACELLULARIS* INFECTIONS, THE CAUSE OF PORCINE PROLIFERATIVE ENTEROPATHY ('ILEITIS') IN THE PIG

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Summary

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

In the United Kingdom, 67% of antimicrobials are delivered via the feed and at least a similar proportion is administered to pigs. Several important families of antibiotics are used to treat and prevent ileitis, including the tetracyclines, macrolides, lincosamides and pleuromutilins. Surprisingly, there is little published information on the pharmacokinetics of these products in the intestines of pigs and especially in the ileum.

The purpose of this paper was to establish a baseline model for antibiotic concentrations that are likely to be achieved in the ileal contents. These could then be related to the pharmacodynamic activity of the antibiotics, in particular to the intracellular minimum inhibitory concentrations (MICs) against L. intracellularis, which were established in the 1990s and correlate these with clinical studies. Pharmacokinetic/pharmacodynamic relationship analysis is a powerful tool, not only to predict dose rates and intervals, but also, as in this case, how antimicrobials are likely to work against target pathogenic bacteria and treat infectious disease.

In the paper, the ileal contents concentration (ICCs) for a number of antibiotics are estimated, but deficiencies in the determination of the intracellular MICs are highlighted. The ICCs do appear to correlate with the inhibitory activity of the antibiotics against L. intracellularis in the intracellular cell culture system, but due to the deficiencies in the data, more precise correlations cannot be made.

Introduction

The prevalence of *Lawsonia intracellularis* infection on farms in the United Kingdom and Ireland is high, at 94.9% (Mortimer *et al,* 2000) and 62.2% of the finishing pigs tested were serologically positive, using a relatively sensitive immuno-fluorescent antibody test. This demonstrates that a large number of animals are exposed to the organism and may suffer from porcine proliferative enteropathy ('ileitis'), either the acute haemorrhagic form in finishers or the more chronic form in growers, which can have a severely damaging effect on growth performance and productivity.

Lesions of cell proliferation primarily affect the terminal ileum, but may extend into the caecum and proximal colon. Characteristic lesions of over 1.5 meters long have been measured in artificial infection studies and the length of the lesion has been directly linked to growth rate depression (Winkelman, 1999).

Several groups of antimicrobials have been shown to have activity against *L. intracellularis*, such as the macrolides, tetracyclines, lincosamides and pleuromutilins (McOrist *et al*, 1995), using a rat intestinal epithelial cell culture to determine intracellular minimum inhibitory concentrations (MICs) and many of these products now have therapeutic claims against this bacterium. However, following the introduction of the European Guideline (EMEA/CVMP, 2001) for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances, there are still question marks over the pharmacokinetic/pharmacodynamic (PK/PD) relationships associated between ileal contents concentrations (ICCs), intracellular MICs and clinical efficacy results. Burch (2003) examined the PK/PD relationships for lincomycin and demonstrated that the ICC gave a good correlation with intracellular inhibition (not necessarily MIC) and clinical effect.

It is the purpose of this paper to examine these relationships further and relate them to other antimicrobials that may be used for the therapy of ileitis.

Pharmacokinetics

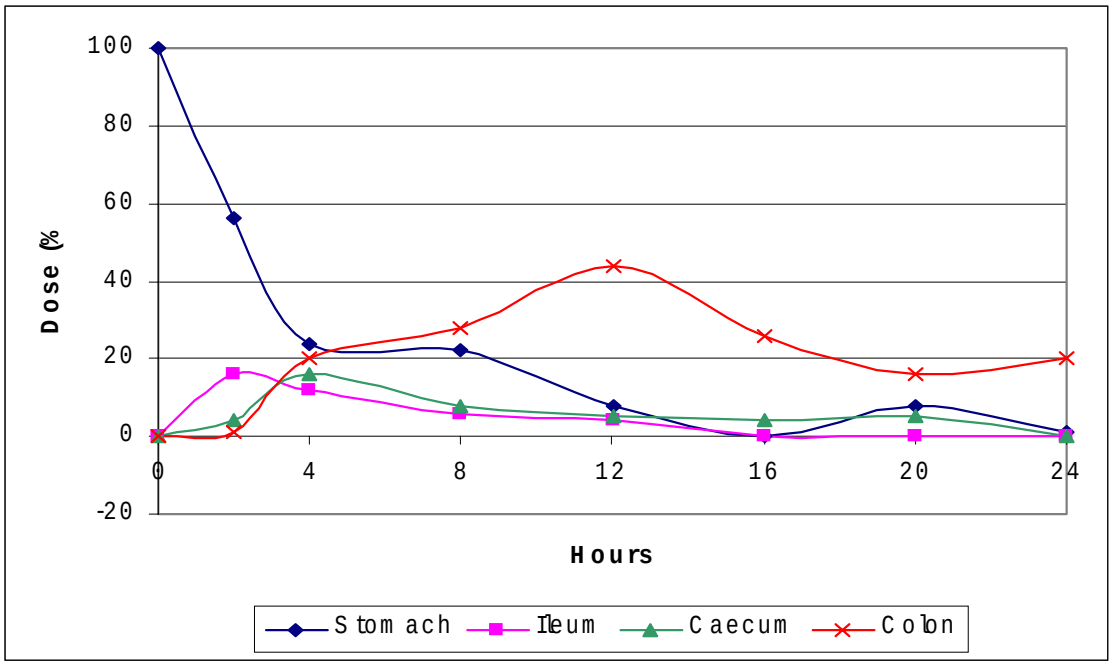
Although antibiotic therapy, given by the oral route in feed, accounts for 67% of all antimicrobial use in the UK (VMD, 2004), there is limited data available on the concentrations achieved in the intestine of the pig to treat various infections.

Clemens *et al* (1975) looked at the passage of food along the intestinal tract of adult pigs using liquid markers (polyethylene glycol and chromium-labelled ethylenediaminetetraacetic acid), which was dosed by stomach tube mid-meal to

pigs fed every 12 hours. The flow and percentage of dose in various sections of the gut were determined following sequential slaughter of the pigs at 0, 2, 4, 8, 12, 16, 20, 24 and 38 hours.

The fluid markers were observed to leave the stomach quite rapidly, with no apparent accumulation in the small intestine, caecum or proximal colon, but reached a peak of 60% of dose in the ascending colon, 12 hours after feeding. This gradually passed on over the next 24 hours to the descending colon, where peak concentrations of 52% were reached 38 hours after dosing (See Graph 1).

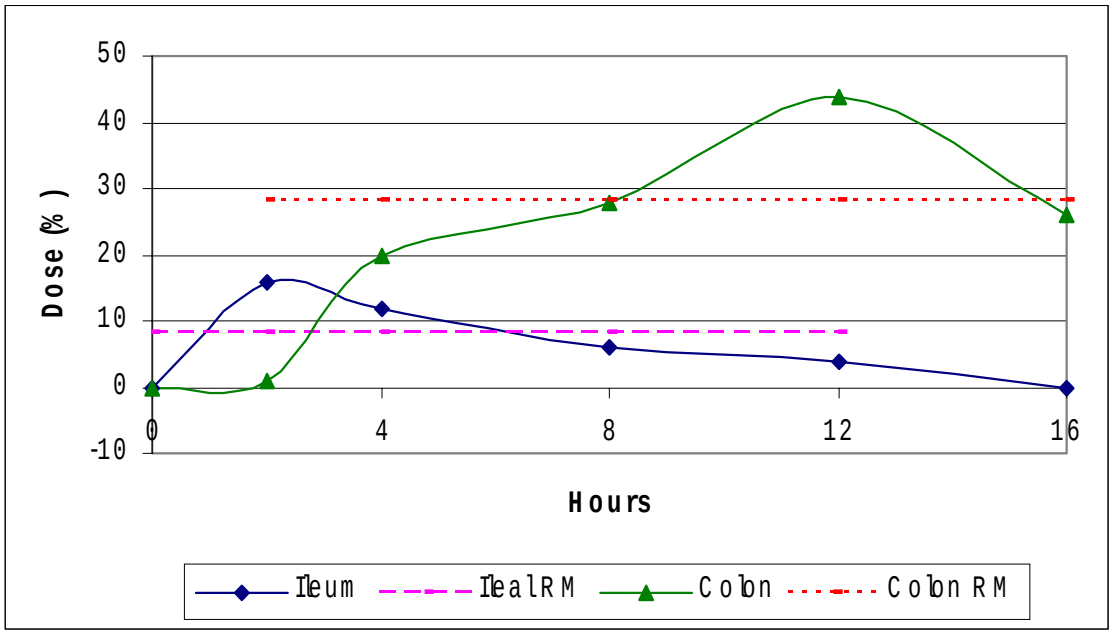
Graph 1 - Dose (%) found in parts of the gut at certain time points after administration



This represents a fairly dynamic flow situation in the small intestine, but a much slower passage in the colon. In addition, in the normal pig it is fed twice daily or *ad libitum*, i.e. several smaller meals, so the flow becomes more or less continuous along the small intestine over a 24 hour period, as well as the colon, but at a much slower rate.

On this basis, the curves represented by the concentration of the markers in the ileum and colon would give an approximate relationship of the concentration of active substances, like antimicrobials, in these sections of the gut (see Graph 2).

Graph 2 - Dose (%) found in the ileum and caecum at various time points after administration and the calculated rolling mean figure over a 12 hour period for each



By taking the area under the curve for 0-12 hours (AUC 12) for the ileum and the AUC for 2-14 hours for the colon, the rolling means can be calculated and the concentration ratio of the ileal contents to the colon contents can be estimated (see Table 1).

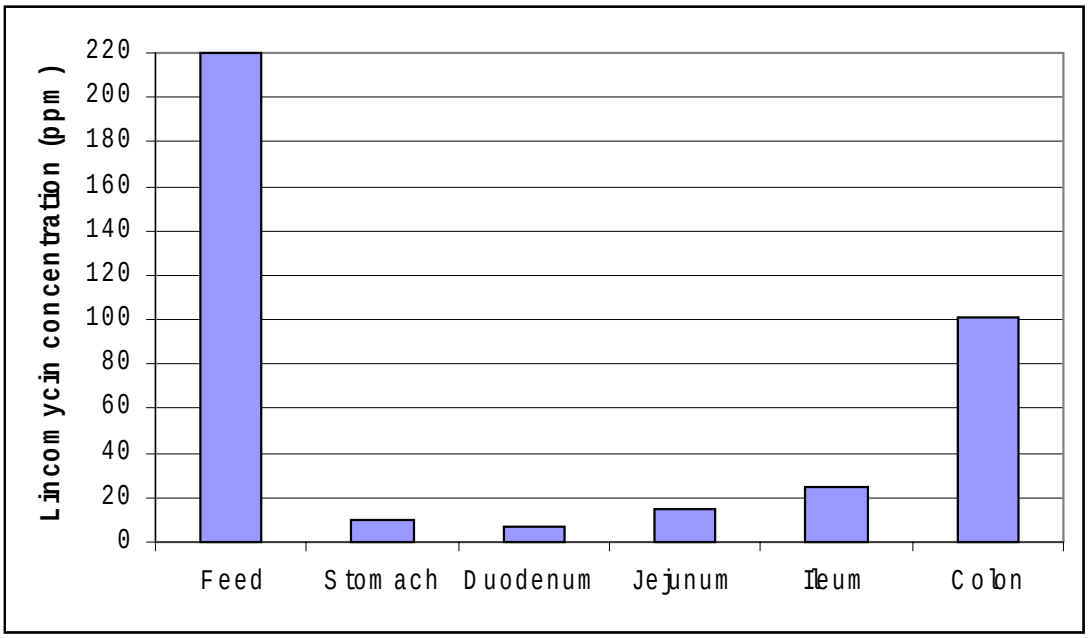
Table 1 - Pharmacokinetic relationships between the colon and ileum using a marker substance

	Ileum (0-12 hours)	**Colon (2-14hours)**
Area under the curve (AUC 12 hours)	100	340
Rolling mean concentration 12 hours	8.3	28.3
Ileal/colonic concentration ratio	0.29 (1: 3.4)	-

From this it can be seen that there is an approximate 1:3.4 relationship between the ileal contents and the colon contents.

A similar pattern was reported by DeGeeter *et al* (1980) following the feeding of lincomycin at 220 ppm in the feed over 23 days to 29.5 kg pigs (see Graph 3). The ileal contents were 25.05µg/g and 101.01µg/g in the colonic contents, giving a ratio of 0.25 or 1:4. The concentrations found in the other sections of the small intestine tend to confirm the findings of Clemens *et al* (1975) that the flow is more rapid and dynamic than in the colon, where it is slower and products concentrate as water is removed. A relatively steady state appears to exist after feeding for several days

Graph 3 - Concentrations of lincomycin in various parts of the gut following administration in the feed at 220ppm for 23 days



It was also of interest in this study, that the concentrations in the tissues of the various parts of the gut were also recorded and, in general, these were quite low in comparison with the contents (see Table 2).

Table 2 - Comparative concentrations of lincomycin in various parts of the gut for both contents and gut wall tissues

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

There is little available data on the concentrations of products in the colon, let alone in the ileum, hence the interest to develop a relationship model with the colonic contents. Burch (2005) described or estimated the colonic contents for a number of antimicrobials.

Table 3 - Colonic contents concentrations (µg/g) of various antibiotics at various in feed levels (ppm)

Antibiotic/*concentration*	**Levels in feed (ppm)/*Concentrations in colon (µg/g)***		
Valnemulin	200	75	25
Concentration	*5.2*	*1.68*	*0.56-0.65(0.61)E*
Tiamulin	220	110	44
Concentration	*8.05*	*2.84*	*1.12-1.61(1.37)E*
Lincomycin	220	110	44
Concentration	*101*	*34.5*	*13.8-20.2(17.0)E*
Tylosin	200	100	40
Concentration	*100E*	*50E*	*20E*
Acetylisovaleryltylosin	200	100	50
Concentration	*>10-35(22.5)E*	*>5-17.5(11.3)E*	*>2.5-8.8(5.5)E*

E – estimate – proportionately related to reported values

The ileal contents concentration (ICC) was estimated by multiplying the colon contents concentration by 0.29.

Table 4 - Ileal contents concentrations (µg/g) of various antibiotics at various in-feed levels (ppm) (CCC x 0.29)

Antibiotic/*concentration*	**Levels in feed (ppm)/*Concentrations in ileum (µg/g)***		
Valnemulin	200	75	25
Concentration	*1.51*	*0.49*	*0.17-0.19(0.18)E*
Tiamulin	220	110	44
Concentration	*2.33*	*0.82*	*0.32-0.47(0.40)E*
Lincomycin	220	110	44
Concentration	*29.29*	*10.01*	*4.0-5.86(4.93)E*
Tylosin	200	100	40
Concentration	*29E*	*14.5E*	*5.8E*
Acetylisovaleryltylosin	200	100	50
Concentration	*>2.9-10.15(6.53)E*	*>1.45-5.08(3.28)E*	*>0.73-2.55(1.60)E*

E – estimate – proportionately related to reported values

The pleuromutilins, valnemulin and tiamulin appear to achieve relatively low concentrations in the ileum in comparison with lincomycin and tylosin and acetylisovaleryltylosin is in-between. Spectinomycin as part of Lincospectin is

also approved for the treatment of ileitis at 44ppm of each product (88ppm combined). Spectinomycin is poorly absorbed from the gut and from a radio-labelled study (EMEA/MRL, 2000), 79% remained in the gut, 4.5% was excreted via the urine and 0.05% found in the faeces, 12 hours post-dosing. An approximate concentration of the product can be calculated in the colon, e.g. 79-95.5% (87.25%) of the concentration in feed times 44ppm x 2.5 equals 96ppm x 0.29, to give the concentration in the ileum, equals 27.8ppm or µg/g.

Pharmacodynamics

McOrist *et al* (1995) described some classical work, looking at the extracellular and intracellular MICs of various antimicrobials against a number of isolates of *L. intracellularis*. To determine the extracellular MICs, the antimicrobials were added to the medium, which contained the infectious agent, which was used to infect the cell culture. This was replaced after 24 hours with fresh medium which did not contain antimicrobials. To determine the intracellular MIC, the rat intestinal epithelial cell cultures were infected with *L. intracellularis* and the culture medium was replaced after 24 hours with one containing various concentrations of the different antimicrobials to be tested. These were replaced at 48 and 72 hours with new medium containing the antimicrobials (three days total exposure) and then replaced until day 7 with non-medicated medium, when the cultures were examined for heavily infected cells (HIC->30 bacteria/cell) and compared with a non-medicated control. The results were expressed as a percentage of HICs in treated cultures divided by the mean HICs in untreated cultures to give a percentage inhibition. A data matrix comprising the HIC results for each strain (1-3 strains tested) and each antimicrobial was set up, and the results were expressed from the combined results. The extracellular and intracellular MIC for each antimicrobial was determined when the inhibition was greater than 99%. The intracellular MIC for Valnemulin was reported by McOrist *et al* (1998). In a further paper, McOrist and Gebhart (1995) also described a minimum bactericidal concentration (MBC) test for a limited number of antibiotics against *L. intracellularis*. It followed a similar method, but only exposed the cultures to the antibiotic for 1 day, 24 hours after infection, then the medium was replaced with untreated medium. The cultures were then re-grown in medium without antibiotic for a further 5 days to see there was no further growth of the organism. The results are summarised in Table 5.

Table 5 - Intracellular MIC and MBC (<99% inhibition) for a number of isolates of *L. intracellularis* against a number of antibiotics

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

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

Mackie (1996), one of the original co-authors in McOrist *et al* (1995), described in her Master's Thesis the individual results of the strains tested. Not all of the strains were titrated to their lowest limits, so an actual MIC could not easily be determined for each isolate. Additionally, the assay method end point of determining a figure for a MIC is really an arbitrary microbiological standard (Burch, 2003). As the method is effectively a bio-model, measuring the concentration of the antimicrobial in the bathing medium, which will penetrate the enterocyte and inhibit the Lawsonia may be too restrictive. A more suitable term would be inhibitory extracellular concentration (IEC) of an antimicrobial which will inhibit the growth of *L. intracellularis* inside the enterocyte.

Table 6 - Inhibitory extracellular concentration (IEC) (intracellular MIC) (*extracellular MIC*) effect and bactericidal extracellular concentration (BEC) effect of various antibiotics on various strains of *L. intracellularis*

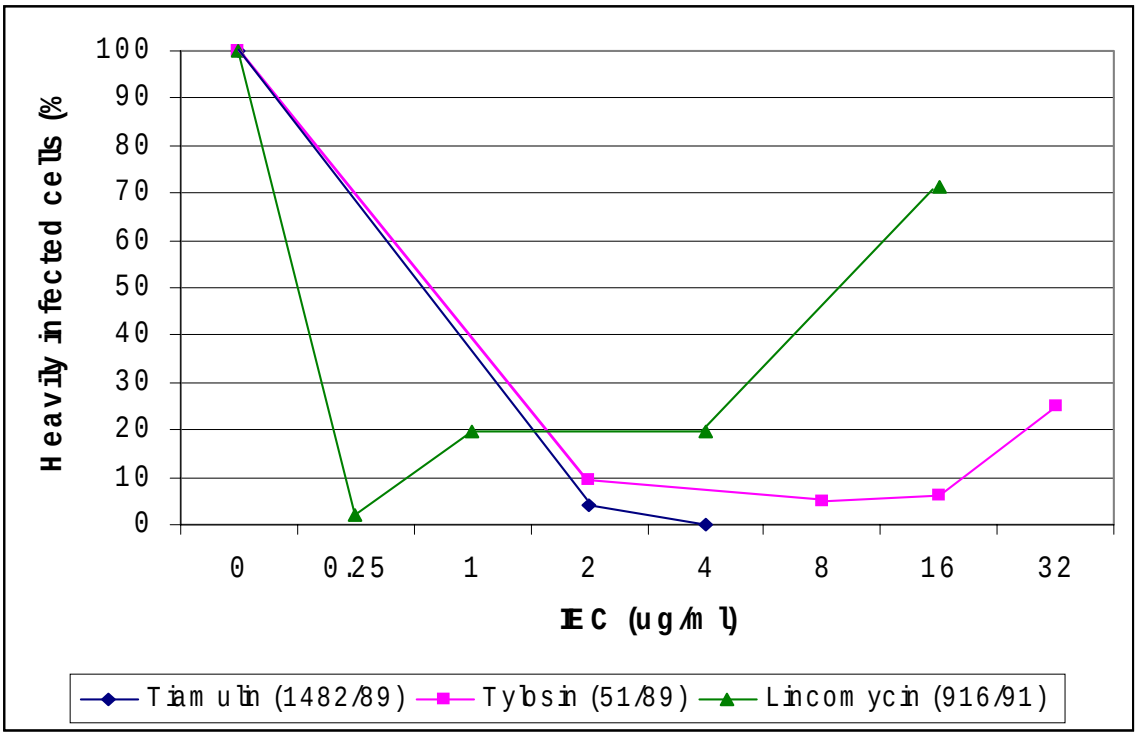
Antibiotic	**IEC (µg/ml) day 2-5**	**Strain 916/91 (NCTC12657)**		**Strain 1482/89 (NCTC12656)**		**Strain 51/89**	
		iMIC	*eMIC*	iMIC	*eMIC*	iMIC	*eMIC*
Valnemulin	1 2 4 8	-		100	*100* *100* *100* *100*	- - 99.6 99.7	- - *99.6* *99.4*
Tiamulin	2 4 8	- - 99.4	 *99.4*	96.4 100	*98.4* *100* *96.4*	- - 99.9	- - *99.4*
Lincomycin	0.25 1 4 16 32	98 80.5 80.5 28.6	39.6 7.1 93.5 93.5	- - - 59.5 11.6	- - - *2.1* *23.8*	-	-
Tylosin	2 8 16 32 100	-		- - - - 99.7	- - - - *99.7*	90.6 95 93.8 75 -	*74.8* *77.9* *96.8* *90.6* -
Tilmicosin	0.125 0.5 2 8	- 90 90	 90 90	-		100 98.5 99.2 98.5	*97.6* *97.6* *100* *100*
Spectinomycin	16 64	36 100	100 97.7	-	-	-	-
Antibiotic	BEC (µg/ml) day 2 only	Strain 916/91 (NCTC12657)		Strain 1482/89 (NCTC12656)		Strain LR189/5/83	
Tiamulin	2	100		-		100	
Tylosin	2 4	-		99.4 100		-	

There are only minor differences between the early applications of most antibiotics at the time of infection (*extracellular MIC*), before the Lawsonia have penetrated into the cell and those once the cell has been infected and treated 24 hours later (intracellular MIC) for 3 days. However, lincomycin showed a lower efficacy in early treatment, but spectinomycin showed a marked improvement, presumably because it has a more bactericidal activity against the unprotected

organism and poor cell penetration subsequently. The internalization of the organism into the cell is as little as three hours (Gebhart, 2004) by membrane-bound vacuoles, before release into the cytoplasm.

It is difficult to make precise interpretations of the IEC or BEC, as the lowest concentrations have not been determined. There are different patterns observed by different antibiotics. Valnemulin, tiamulin and spectinomycin appear to show a dose-related inhibition. The tylosin and tilmicosin patterns of inhibition are rather flat and complete inhibition is not always achieved; whereas lincomycin's inhibition decreases with concentration (see Graph 3). The BECs are lower than the IECs, which is surprising, but really demonstrates the importance of using a greater range of concentrations of antibiotic to determine the inhibitory or bactericidal level.

Graph 4 - Comparative intracellular inhibition patterns with increasing concentrations of antibiotic in the cell culture medium (Intracellular MICs)



If the IEC interpretation is lifted to 90% inhibition rather than 99% (which is very restrictive in experimental terms and the results achieved) and also the lowest concentration figure used for a single isolate, a different inhibitory picture emerges, which, apart from spectinomycin, is substantially lower than originally reported.

Table 7- Comparison of inhibitory extracellular concentrations for various antibiotics giving a 90 or 99% inhibition

Antibiotic	IEC (µg/ml) 99% inhibition (1-3 strains) McOrist *et al,* 1995/98	IEC (µg/ml) 90% inhibition (1 strain - lowest) Mackie, 1996
Valnemulin	<2	≤1
Tiamulin	4	≤2
Lincomycin	32	≤0.25
Tylosin	64	≤2
Tilmicosin	2	≤0.125
Spectinomycin	32	≤64

Pharmacokinetic/pharmacodynamic relationships

When the ileal contents concentration (ICC) is compared with the IEC90%, a relatively similar figure is achieved, except for spectinomycin.

Table 8 - Comparison of inhibitory extracellular concentrations 90% and ileal contents concentration of various antibiotics

Antibiotic	IEC (µg/ml) 90% inhibition (1 strain - lowest) Mackie, 1996	Level in feed (ppm)	ICC (µg/ml)*
Valnemulin	≤1	75 25	0.54 0.18
Tiamulin	≤2	150 50	1.36 0.45
Lincomycin	≤0.25	110 44 22	12.33 4.93 2.47
Tylosin	≤2	100 40	14.5 5.8
Spectinomycin	≤64	44 22	27.8 13.9

* Extrapolated upwards from mean values or estimates at various data points to reduce variation.

As the lowest IEC figures have not been identified, as a sufficient range of antibiotic concentrations were not investigated, it is not possible to determine an ICC/IEC ratio. However, the ileal contents concentration (ICC) figures are approaching the inhibitory extracellular concentration (IEC90) or exceed them, in the case of lincomycin and tylosin, suggesting that there is a relatively close relationship. No gut concentrations can be found for tilmicosin, but its very low IEC90 figures would suggest *L. intracellularis* would be highly susceptible. There

are no published IEC90 figures for acetylisovaleryltylosin, but might be in the order of 1.6-3.3µg/ml, based on efficacy data.

Clinical effects of various antibiotics on ileitis

Tiamulin

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

In the infected control, 6/7 pigs showed gross lesions and all showed histological lesions in the ileum and 5/7 in the caecum. Both the 50ppm tiamulin for prevention and 150ppm treated pigs showed no evidence of gross or histopathological lesions or the presence of stained organisms. The bactericidal extracellular concentration (BEC) of tiamulin against this strain was ≤2µg/ml and the estimated intestinal contents concentration was approximately 1.36µg/ml for 150ppm and 0.45µg/ml for 50ppm, suggesting that the true IEC is probably around 0.45µg/ml.

Tylosin

McOrist *et al* (1997) carried out a similar trial with tylosin, using the same infected cell culture with *L. intracellularis* (strain LR189/5/83.1). Tylosin was given in the feed at 40ppm and 100ppm, four days prior to infection and for a further 16 days after infection when the levels were dropped to 20 and 40ppm respectively, for the remaining 12 days. A further group was treated seven days post-infection for 21 days, when the trial was terminated and the pigs autopsied.

None of the pigs on the tylosin prevention or treatment programmes showed any gross or histopathological signs of infection with *L. intracellularis.* The infected, untreated control showed gross lesions in 5/8 pigs and histopathological lesions in 7/8 in the ileum and 3/8 in the caecum. It can be concluded that tylosin at 40ppm and above and 100ppm were effective in the prevention and treatment of ileitis. The MIC for this particular strain is not

available, but if this is representative, then the intracellular MIC 99% figure of 62µg/ml is not a realistic figure based in ICCs and that the IEC90 or BEC at ≤2 is more representative. Breakpoint work by Burch (2005) for *Brachyspira hyodysenteriae* suggests colon contents concentrations for tylosin 100ppm may be closer to 16µg/ml and then the ICC is likely to be around 1.84-4.6µg/ml for 40 and 100ppm tylosin, respectively, which fits quite well.

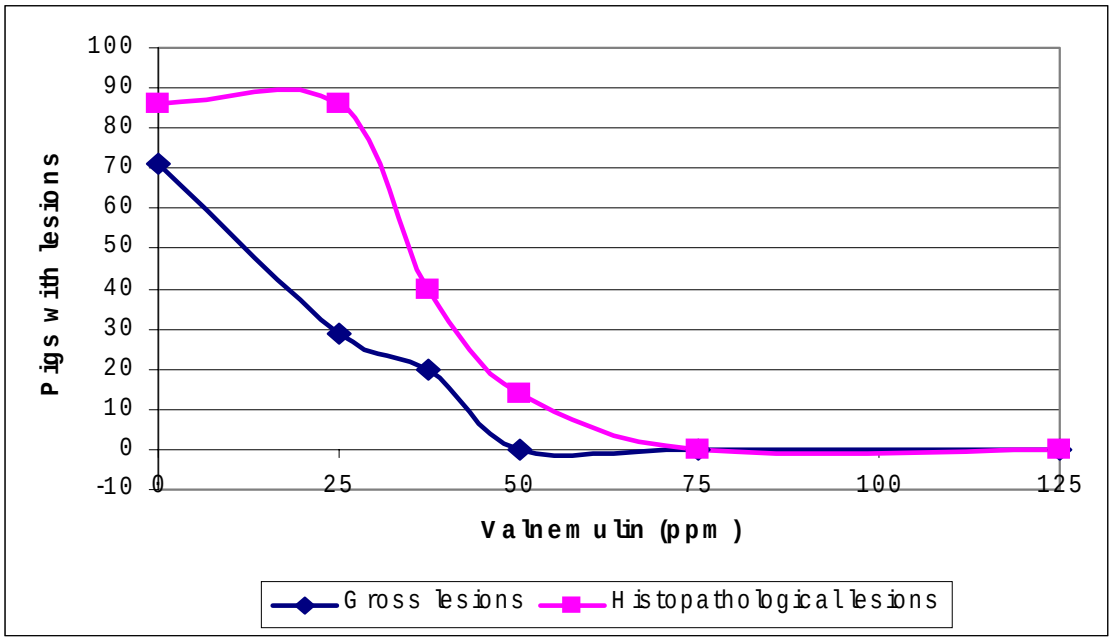
Valnemulin

In a third artificial challenge study, McOrist *et al* (1998) looked at a range of doses of valnemulin in feed for the prevention and treatment of ileitis. The same challenge strain of *L. intracellularis* (strain LR189/5/83.1) was used. The medication for prevention at 25, 37.5 and 50ppm started two days before challenge and continued for a further 21 days. Treatment was administered seven days post-challenge for 14 days when the trial was terminated and the pigs necropsied. The results are summarised in Table 9.

Table 9 - Dose-titration study to evaluate valnemulin for the prevention and treatment of an artificial challenge with *L. intracellularis*

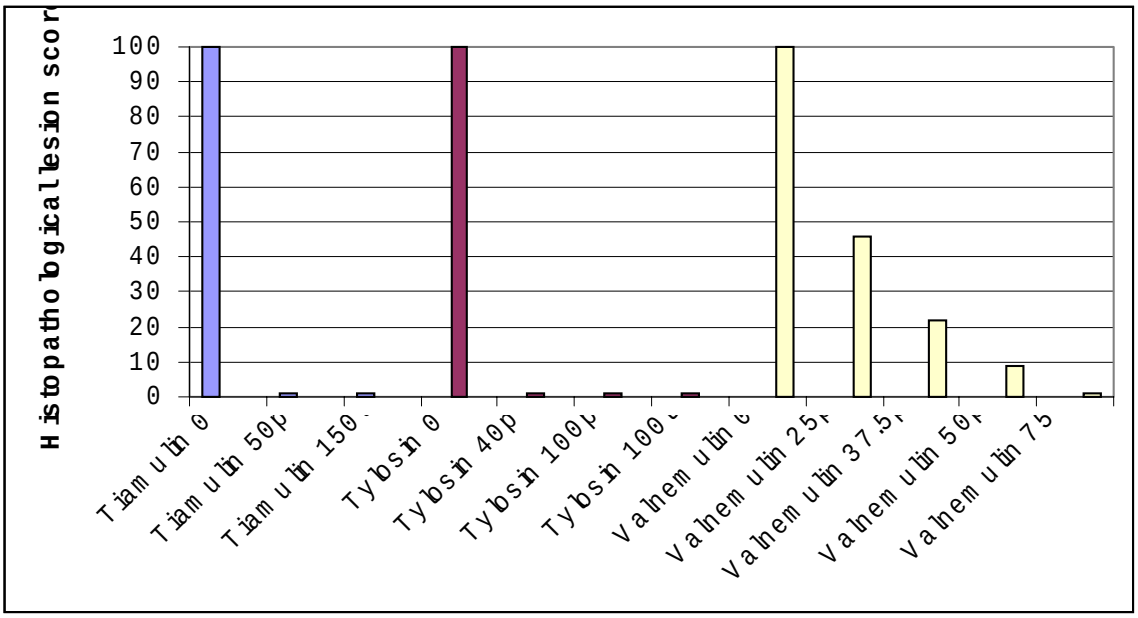
Valnemulin (ppm)	**No. pigs gross lesions**	**No. pigs histopathological lesions**	**Histopathological lesion score (%)**
0	5/7	6/7	100
25 (prevention)	2/7	6/7	46
37.5 (prevention)	1/5	2/5	22
50 (prevention)	0/7	1/7	9
75 (treatment)	0/7	0/7	0
125 (treatment)	0/7	0/7	0

Graph 5 - Effect of valnemulin for prevention and treatment on gross and histopathological lesions of ileitis



There was a dose/effect with valnemulin between 25-50ppm for the prevention of ileitis. Gross lesions were not seen at 50ppm, but histopathological lesions could be seen in 1/7 pigs. Both treatment levels of 75 and 125ppm eliminated histopathological lesions of *L. intracellularis*. This suggests that the IEC90 and BEC is approximately 0.54µg/ml, based on ICC levels and this is supported by the intracellular MIC findings of Mackie (1996) who found it to be ≤1µg/ml, but for strain 1482/89.

Graph 6 - Comparative efficacy of various antimicrobials in the prevention (p) and treatment (t) of ileitis using the McOrist challenge model



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

Table 10 - Efficacy of valnemulin for the prevention of ileitis

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

* p= <0.05

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

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

Lincomycin

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

Table 11 - Efficacy of lincomycin and tylosin for the prevention of ileitis

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

* p= <0.05

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

Acetylisovaleryltylosin

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

Table 12 - Efficacy of acetylisovaleryltylosin and tylosin for the treatment of ileitis

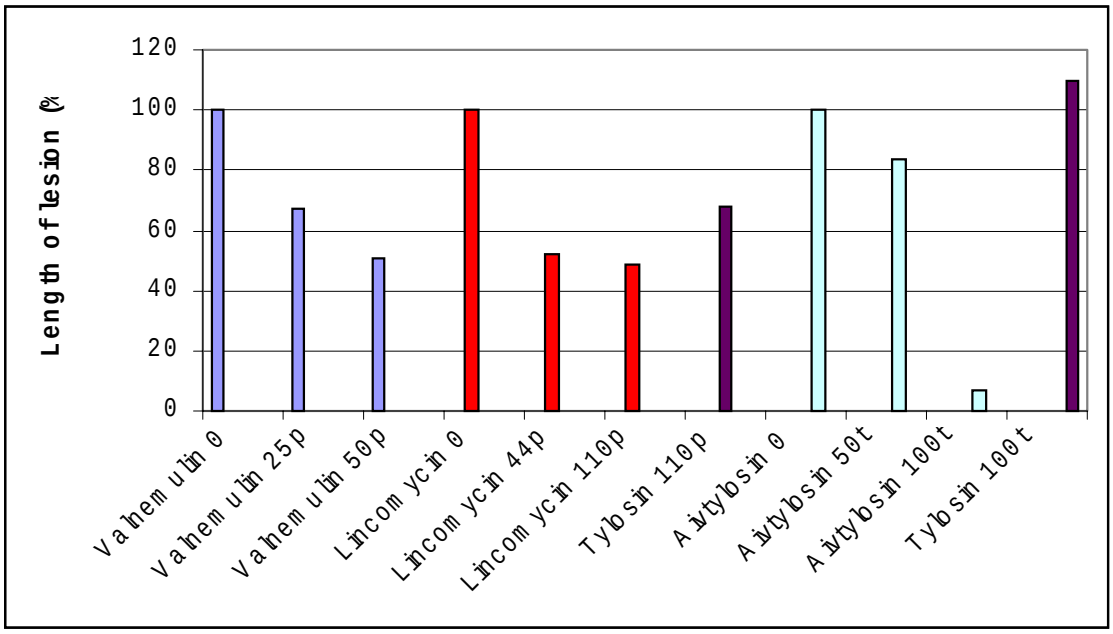
Treatment group (ppm)	Mortality (%)	Intestinal lesion length (cm)
Acetylisovaleryltylosin 0	15	109.5 (-)
Acetylisovaleryltylosin 50	13.3	91.9 (-16%)
Acetylisovaleryltylosin 100	0	8.0 (-93%)*
Tylosin 100	16.7	120.1 (+10%)

* p= <0.001

In this study, with a heavy infectious challenge, tylosin at 100ppm performed less well than the untreated controls by the parameters of mortality and lesion length, which is surprising. Acetylisovaleryltylosin at 50ppm showed only modest improvements over the untreated controls, but at 100ppm there was no mortality and lesion length was reduced by 93%, which is exceptional using the Winkelman challenge model. In addition, the length of medication was half that of tylosin (10 and 21 days respectively) so a strong bactericidal effect can be anticipated at this level in the feed, suggesting the BEC is around 3.28µg/ml (range 1.45-5.08µg/ml).

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

Graph 7 - Comparative efficacy of various antibiotics in the prevention (p) and treatment (t) of ileitis using the Winkelman challenge model



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

Discussion and Conclusions

With the increasing requirement to use PK/PD relationships and modelling as supporting evidence for product approval in the EU, it is useful to determine some of the baselines for its use for individual diseases and with individual antibiotics. From this work, a PK relationship can be established between colon contents and ileal contents concentration, where little published information is available. Although the Clemens *et al* (1975) technique is highly useful, it does relate to a single in feed dose rather than a prolonged course of treatment and it is possible the concentration in the colonic contents is higher after repeated dosing. The work was also in adult animals and may be slower than in younger pigs and disease and diarrhoea especially, may have an impact on rates of flow. However, personal observation on unpublished data for a number of antibiotics does support the relationship data.

Using PK/PD relationships has also highlighted some disparities in the pharmacodynamic data such as the intracellular MIC for tylosin at 64µg/ml, when ileal concentrations did not reach it, yet it is probably the most commonly used product for the treatment of ileitis. This then encouraged further examination of the data and how it was derived. The intracellular growth of *L. intracellularis* and the development of the MIC/MBC models was outstanding work and apparently very time-consuming. Some of the extrapolations, however, may have resulted in some misleading assumptions about the relative and absolute activity of some of the antimicrobials used. The Mackie (1996) work highlights that the lowest inhibitory endpoint was not established and an over-estimation of the reported MIC has been made.

In fact, the inhibitory extracellular concentration (IEC) determinations to establish the MICs were highly effective models in predicting the likely efficacy of antibiotics to inhibit or treat the Lawsonia infection. If the lower endpoints had been determined, more precise predictions could be made. The MIC 99 figure used also causes some confusion, because of the sensitivity of the intracellular model infection, as there is sometimes a lot of variation between the concentrations and the inhibitory effect observed. This is frequently more than 1%, which can have a substantial impact on the MIC figure derived. The concentration/inhibitory effect curve is quite sufficient to determine the IEC 90, but not the MIC 99 in many cases and this gives a better correlation with clinical inhibition of the disease. The observation that spectinomycin had a lower extracellular MIC than intracellular

MIC in comparison with lincomycin was also of interest. This suggests that the spectinomycin component of Lincospectin (Pfizer) is mainly acting on extracellular Lawsonia, whereas the lincomycin is mainly acting on those in the cell.

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

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