

PHARMACOKINETICS, MINIMAL INHIBITORY CONCENTRATIONS AND EFFICACY MODEL RELATIONSHIPS FOR SOME GUT INFECTIONS IN PIGS

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Summary

Guidelines have been recently introduced in the European Union for antimicrobial products, to assess their potential for resistance development and for the demonstration of their efficacy using therapeutic regimens to minimise the risk of selecting antimicrobial resistance. A key part of this is the use of pharmacokinetic and pharmacodynamic analysis. Much work has been reported on the use of concentration-dependent bactericidal products such as the fluoroquinolones and aminoglycosides administered by injection, in both man and animals, for systemic or respiratory infections. In contrast, little work has been reported on bacteriostatic compounds administered orally for enteric infections, which is the most common route for pigs. Two examples have been described - lincomycin for controlling Lawsonia intracellularis infections in the pig (porcine intestinal adenomatosis) and valnemulin for the prevention and treatment of Brachyspira hyodysenteriae (swine dysentery). Predicted concentrations of lincomycin in the ileum in relation to the intracellular inhibitory concentrations (IIC) of lincomycin against L. intracellularis corresponded very closely with the clinical responses found in challenge studies. This may be due to the IIC study being a bio-model itself. With valnemulin, the concentrations in colonic contents had to be nine times higher than the minimum inhibitory concentration for B. hyodysenteriae to achieve preventative inhibition in a challenge study and 90 times higher to achieve bacterial elimination in a treatment study, as other factors come into play. It demonstrates that prevention is a legitimate claim and not just an excuse for growth promotion and the reliance on treatment regimes only, might actually encourage B. hyodysenteriae resistance development, as seen in Germany.

Introduction

Recently, guidelines for antimicrobial products have been introduced in the European Union (EU) with regard to resistance development (EMEA/CVMP/244/01) and efficacy (EMEA/CVMP/627/01). This is an attempt to reduce the development of resistant bacteria in animals and also to reduce the

potential spread to man. An important part of these guidelines is the use of the pharmacokinetics (PK) and pharmacodynamics (PD) of the antimicrobial to model and confirm an expected dose. In the main, this is a suitable concentration to rapidly kill the organism and remove the opportunity for it to develop resistance. Much work in man and animals has thus focused necessarily on the concentration-dependent bactericidal injectable antimicrobials such as the fluoroquinolones and the aminoglycosides. This is in immuno-compromised human patients and in functional immune system animals (Sarasola *et al*, 2002). Very little PK/PD modelling work has been described on primarily bacteriostatic antimicrobials administered orally, mainly in feed, which are of the most antimicrobial use in pigs. This paper examines the parameters that are useful for making an assessment of efficacy using model and dose confirmation studies with publicly available data for two major gut infections ileitis (porcine intestinal adenomatosis) and swine dysentery.

In-feed administration of antimicrobials – general pharmacokinetic considerations

When an antimicrobial is given in feed, assuming it is not absorbed and not broken down, a concentration in the feed of 100ppm will be excreted in faeces at about 250ppm as one kg of feed is converted to 0.4kg of faeces. So, there is overall, a concentrating effect during the passage through the intestine. However, before then, food in the stomach is mixed with liquids, e.g. saliva, acid secretions, mucus and possibly liquids, such as water or whey, causing a dilution effect. Gastric emptying half times are about two hours for liquids and eight hours for solids (Argenzio and Monteiro-Riviere, 2001) and passage down the small intestine takes a further 12 hours for solids. Additional fluids are added via secretion and bile and digestive enzymes, causing further dilution and, at the same time, absorption of the antimicrobial may be occurring, further reducing the concentration. Break down of the product by digestive enzymes may reduce the concentration further but after absorption, metabolism in the liver and excretion via the bile, there may be an increased concentration effect. Overall, there is usually a dilution effect in the small intestine, which may start to reduce in the ileum as fluids are gradually removed and concentrations increase. The small intestine therefore is basically bathed in the antimicrobial for almost 12 hours after feeding and a pig may have a relatively steady flow and concentration of antimicrobial there. Feed also alters the bioavailability of many compounds when compared with bolus dosing. Frequently they are reduced (Nielsen, 1997) due to the prolonged passage and absorption and continuous metabolism via the liver. For example, lincomycin's bioavailability is reduced from 73% to 41% in unfed and fed pigs respectively.

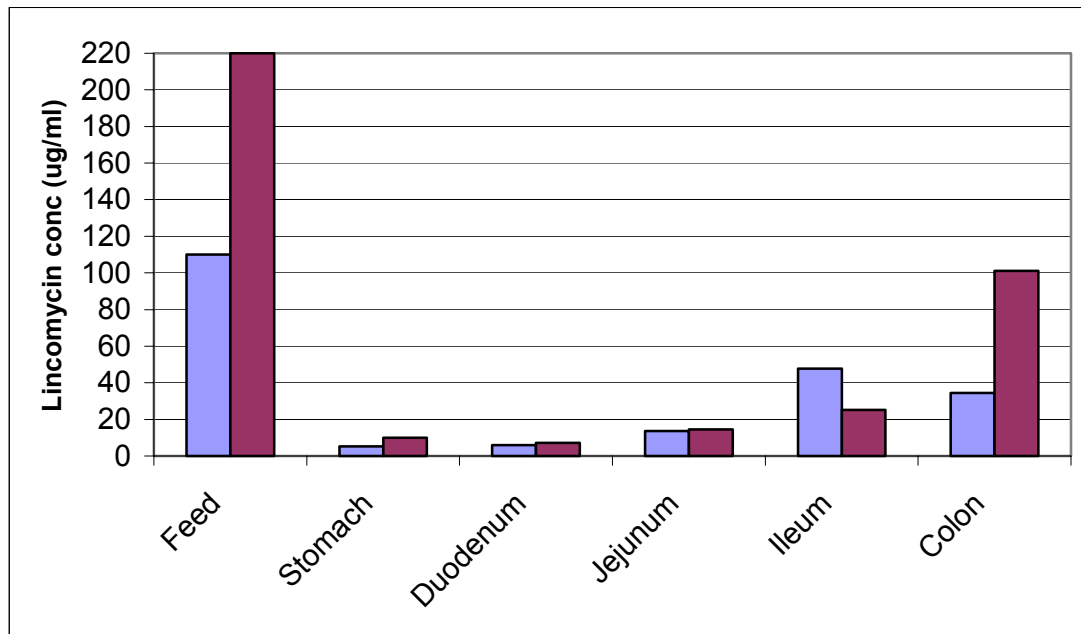
In the colon, the passage of feed is much slower and takes 24-48 hours. There is a concentration effect as liquids are removed. However, there are large numbers of bacteria present that can break down antimicrobials. Faecal binding can also affect drug availability. Each product has its own characteristics and stability and it is necessary to measure the concentrations in the various parts of the intestine to improve predictions of efficacy. This depends on which part of the gut is affected by the organisms of interest.

The minimum inhibitory concentrations (MIC) of the bacteria are required and, ideally, a representative population derived from several member states of the EU is needed to determine the relationship of the effective gut concentration of the product and the MIC 90 (MIC of 90% of the isolates) of the susceptible isolates to forecast the likely efficacy of the compound and dose.

Ileitis – small intestine infection – lincomycin model

The concentrations of lincomycin, in various parts of the pig intestine, have been reported (DeGeeter *et al*, 1980) following the administration in feed at 110 and 220ppm (see Graph 1.)

Graph 1 - Lincomycin concentrations in the gut following feeding 110 and 220ppm



A dilution effect can be seen in the stomach and small intestine, but concentrations increase in the ileum and colon. There seems to be a major discrepancy in the ileal concentrations and this may be a result of the assay

method sensitivity (unreported), the dynamic nature of the small intestine (as the standard deviations are quite high) or due to another unexplained factor.

Table 1 - Lincomycin conc. ($\mu\text{g/ml}$) in the gut contents following feeding at 110 and 220ppm

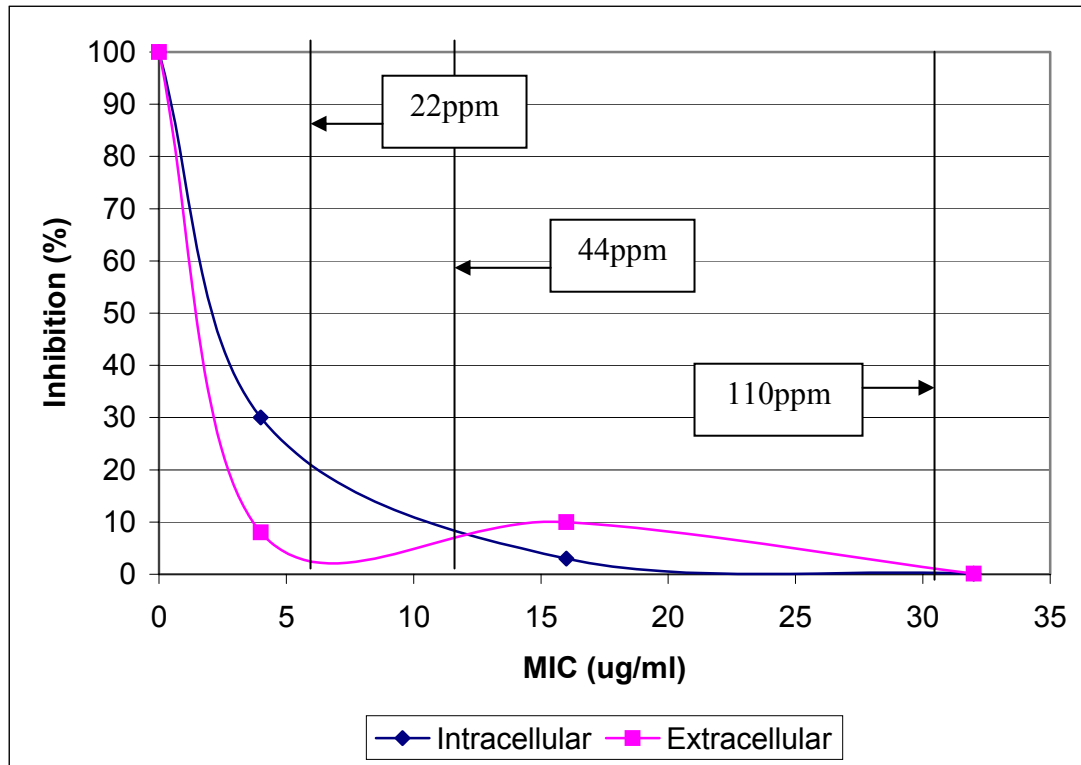
Organ	Lincomycin 110ppm	SD	%	Lincomycin 220ppm	SD	%
Feed	110	-	100	220	-	100
Stomach	5.15	4.95	5.2	9.86	6.85	4.5
Duodenum	5.90	4.97	5.4	7.18	6.40	3.3
Jejunum	13.71	9.90	12.5	14.48	9.36	6.6
Ileum	47.82	21.20	42.9	25.05	10.97	11.4
Colon	34.51	15.28	31.4	101.01	24.64	45.9

A mean percentage figure will be used in the model for ileal concentration of 27% of feed concentration, so 110ppm feed is 30ppm, 44ppm feed is 12ppm and 22ppm feed is 6ppm. The organism causing ileitis is *Lawsonia intracellularis*, which is an obligate intracellular bacterium. It spreads from pig to pig via faecal contamination and therefore has to pass down the intestine and invade a host enterocytes. Usually, this is in the ileum, but the lesions can spread up into the jejunum and down into the caecum and proximal colon. There it grows and causes the typical cellular proliferation associated with the disease.

McOrist *et al* (1995) described an ingenious method of testing antimicrobials and their relative activity in inhibiting the growth and damage of *L. intracellularis* in rat enterocyte cell cultures. They described an intracellular MIC where the infected cell cultures were bathed for 4 days in increasing concentrations of antimicrobial, starting one day after infection. The inhibitory effect was based on the comparison of heavily infected cells (HIC) (>30 bacteria/cell) in an infected control with the antimicrobial-treated cell cultures and expressed as percentage inhibition. In effect, this is a bio-model itself and depends on external concentrations of antimicrobial providing a sufficient gradient for it to penetrate the cell membrane and inhibit the organism, just like gut concentrations of antimicrobial and lesion inhibition. The MIC of 99% HIC inhibition is an arbitrary microbiological standard, which has been used, but as a bio-model itself may be too restrictive clinically and relatively imprecise because of the gaps in dilutions used. The extracellular MIC is more akin to standard microbiological techniques where infected culture medium containing the antimicrobial was added to the cell cultures. After the first day, when the medium was replaced, it contained no antimicrobial. It had to exert its inhibitory/killing effect within 24 hours. *L. intracellularis* is reported to penetrate intestinal cells very quickly to enable survival and this may be a limitation of this method. The PD properties and killing curves of bacteriostatic substances might be too slow. This may be

considered more for prevention than for treatment and in the US it is termed as control. The inhibition curve/concentrations for lincomycin, both intracellularly and extracellularly, against *L. intracellularis* are shown in Graph 2. The estimated ileal concentrations for 110, 44, 22ppm in feed of 30, 12 and 6 ppm respectively have been added.

Graph 2 -Intracellular and extracellular inhibition curves for lincomycin against *L. intracellularis* plus estimated ileal concentrations for 110, 44, and 22ppm in feed



From this, both 44 and 110 ppm would be expected to exert a marked inhibitory effect (greater than 90% inhibition) against *L. intracellularis* contained intracellularly, but 22 ppm would not be so effective for treatment and would exert a preventative effect on extracellular organisms. It is also close to a critically steep part of the curve.

A dose titration study for the prevention of ileitis involving 130 grower pigs in 5 replicates was reported (Winkelman *et al*, 1998; Winkelman, 1999). Pigs were placed on lincomycin at 110, 44, 22 and 0ppm. Unfortunately, the feed analysis showed the 22ppm to be lower than expected, at 5ppm, whereas the others were within normal limits. Tylosin 110ppm acted as a positive control. Four days later they were challenged with a ground up mucosal homogenate that contained high numbers of *L. intracellularis* from previously infected animals and this was

administered orally on two consecutive days. Additionally, prednisolone was administered intramuscularly to enhance the onset of disease. The pigs were treated with lincomycin for a total of 35 days and the results are summarised in Table 2.

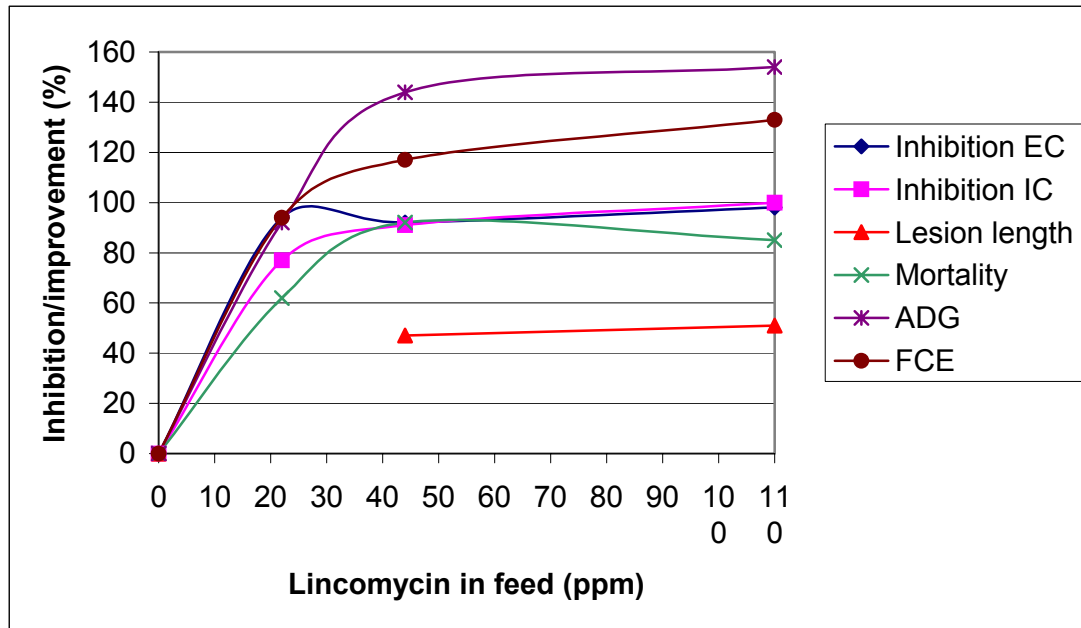
Table 2 - Results of lincomycin in feed prevention study

Treatment (ppm)	ADG (g)	FCE	Lesion length (cm)	Mortality (%)
Untreated infected control	95	0.18	160	52
Lincomycin 22	182	0.35	NR	20
Lincomycin 44	232	0.39	84	4
Lincomycin 110	241	0.42	79	8
Tylosin 110	168	0.32	109	16

This can be considered a relatively aggressive model, as the mortality was 52% in the untreated controls, whereas in field infections it is considerably lower, normally only a few *percent*. It can be considered a severe challenge infection model.

If the improvements of performance (ADG and FCE) and disease (mortality and lesion length) are expressed as percentages and are superimposed on the predictive IC and EC inhibition effects of the varying lincomycin concentrations also expressed as percentage inhibitory effect, an interesting pattern of predictive effect and actual effect can be seen (see Graph 3.)

Graph 3 - Predictive inhibition and actual effect of lincomycin included in feed at 22, 44 and 110ppm



In disease prevention terms, the mortality and inhibition figures were the most similar. Unfortunately, the lincomycin 22ppm figures for lesion length were not recorded. Both curves seemed to plateau at the 44ppm level and there were minor movements up and down at the 110ppm level. Lincomycin at 22 ppm was sub-optimal, although showed a marked reduction in mortality. With regard to performance characteristics of ADG and FCE, again, these tended to plateau at the 44ppm level and, although lower at 22ppm, significant improvements were noted. All were superior to the positive control tylosin at 110ppm. Winkelman (1999) demonstrated a good linear relationship between lesion length and ADG.

The challenge model is very severe and milder models have been used with tylosin at 40ppm and 100ppm for prevention and treatment, giving 100% protective results (McOrist *et al*, 1997). Mortality was not a feature, but lesion length was not described. This may be considered a more sensitive infectious model to use and may give a closer lesion/cell culture inhibition relationship.

Although the data is taken from three different sources and may be considered to have some minor deficiencies and discrepancies, it demonstrates that, overall, a good predictive and actual fit can be achieved by using gut pharmacokinetics and an inhibitory effect on *L. intracellularis* data and linking it to clinical trial work. The IC/EC inhibitory concentration may be considered too restrictive at 99% inhibition, as a good clinical effect may be achieved at lower levels of, say 90%, and in the case of lincomycin, this would be at 12µg/ml rather than the MIC of 32µg/ml, which is numerically substantially different and possibly

misleading, although only just more than one dilution different in microbiological terms.

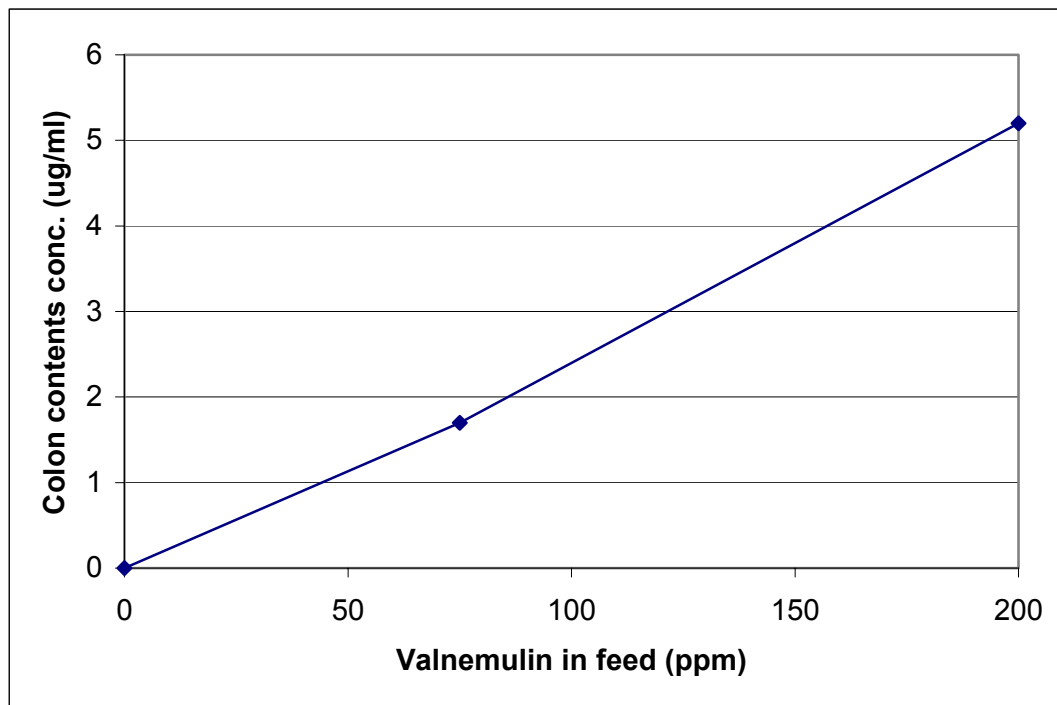
With regard to incomplete kill of the organism and potential resistance development by *L. intracellularis*, it is considered highly unlikely (McOrist; personal communication) as, in part, these are energy dependent processes, which are not available in this type of special intracellular bacteria.

Swine dysentery – large intestine infection – valnemulin model

Valnemulin (Econor – Novartis Animal Health), a pleuromutilin antibiotic, has been shown to have exceptional activity against *Brachyspira hyodysenteriae*, *in vitro*, at levels ranging from 0.0156 – 1.0µg/ml (Moller *et al*, 1996). *B. hyodysenteriae* is primarily a surface living anaerobic bacteria and causes damage to the mucosal cells lining the colon. It also penetrates deep into the crypts in the mucosa and there causes cell necrosis and colitis, resulting in diarrhoea and dysentery in severe cases.

Its concentration in the colon contents has been reported in the product literature, with 200ppm valnemulin in food giving 5.20µg/ml and 75ppm giving 1.68µg/ml. When expressed as a graph, the concentration in the colon is quite linear, so estimations for lower concentrations can be made, e.g. 5, 10, 20ppm would give 0.11, 0.22 and 0.45µg/ml respectively.

Graph 4 - Valnemulin concentrations in the colon contents following feeding at 200 and 75ppm



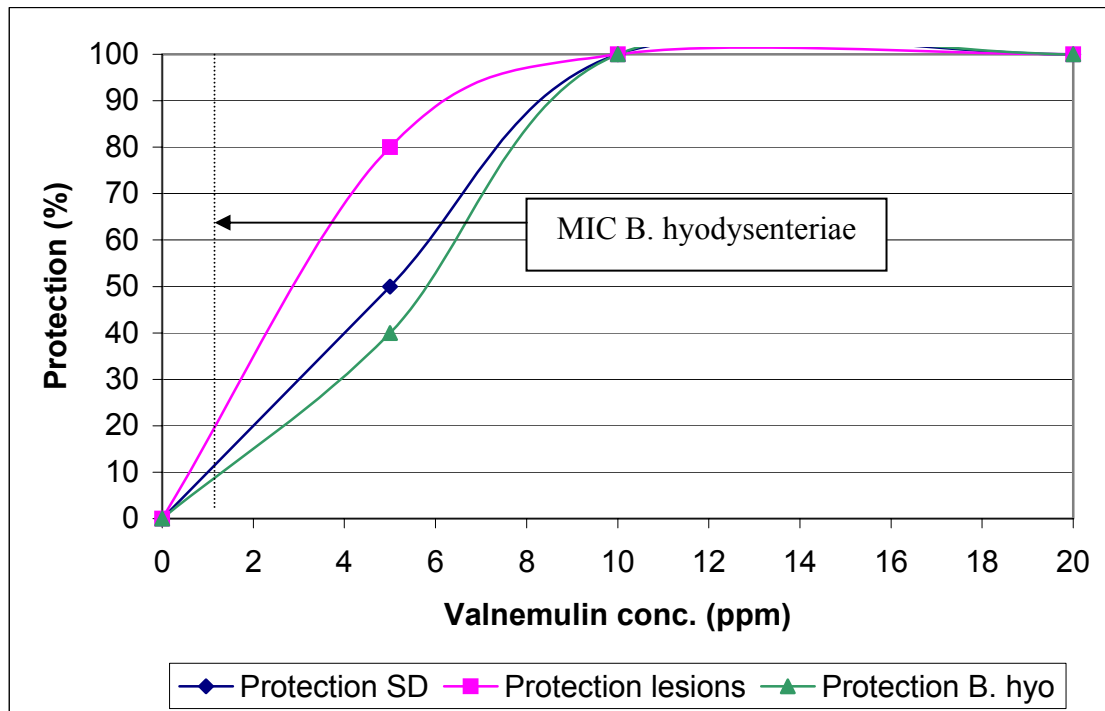
In a prevention study, levels of 5, 10 and 20ppm valnemulin were used and the MIC of the challenge strain was recorded at 0.025 μ g/ml (Burrows *et al*, 1996a). The pigs were challenged with *B. hyodysenteriae* twice on two consecutive days and put onto the medicated feeds the following day. The pigs were sacrificed 21 days after the original challenge and the large intestine was examined for the presence of lesions and mucosal scrapings taken from four areas and cultured for *B. hyodysenteriae*.

Table 3 - Results of the valnemulin dose-titration prevention of swine dysentery study

Treatment group	No. of pigs with dysentery (%)	No. of pigs with gross lesions (%)	No. of pigs <i>B. hyodysenteriae</i> isolated pre- and post- mortem (%)
Untreated infected control	100	100	100
Valnemulin 5ppm	50	20	40
Valnemulin 10ppm	0	0	0
Valnemulin 20ppm	0	0	0

The results were reversed and used as percentage protection and compared with the inclusion rate for valnemulin and the equivalent inclusion for the MIC of the challenge organism (1.1ppm).

Graph 5 - Valnemulin concentration in feed and inhibition of *B. hyodysenteriae*



There is a good correlation between clinical effect and inclusion rate, with 10ppm and above giving a complete bacterial control. This predicted concentration is approximately 9 times the MIC for the challenge strain. Below this, at 4.5 times MIC, there is only an intermediate effect. This is primarily for prevention only, before the bacteria have penetrated deeply into the crypts and become fully colonized.

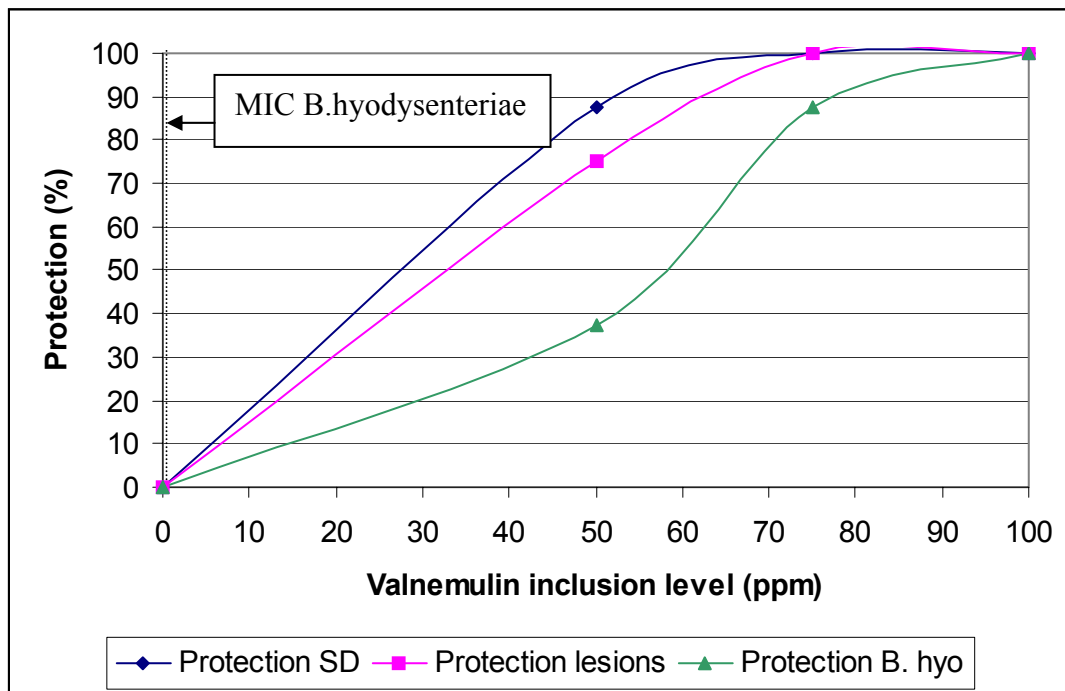
A treatment study using the same challenge strain of *B. hyodysenteriae* was also carried out, but using levels of valnemulin at 50, 75, 100 and 150ppm (Burrows *et al*, 1996b). This was administered to pigs at the onset of clinical disease and fed for 10 days. There was an observation period of a further 2 weeks, to see if there was any recurrence of the disease and the pigs were sacrificed and examined as before. The results are summarized in Table 4.

Table 4 - Results of the valnemulin dose-titration treatment of swine dysentery study

Treatment group	No. of pigs with dysentery or died at the end of study (%)	No. of pigs with gross lesions at autopsy (%)	No. of pigs <i>B. hyodysenteriae</i> isolated at autopsy (%)
Untreated infected control	100	100	100
Valnemulin 50ppm	12.5	25	62.5
Valnemulin 75ppm	0	0	12.5
Valnemulin 100ppm	0	0	0
Valnemulin 150ppm	0	0	0

The results were reversed and used as percentage protection and compared with the inclusion rate for valnemulin and the equivalent inclusion for the MIC of the challenge organism (1.1ppm).

Graph 6 - Valnemulin concentration in feed and inhibition of *B. hyodysenteriae*



Good initial clinical effect was achieved with 50ppm and above, good lesion control was achieved with 75ppm and above, but bacterial cure was successful at 100ppm or 91 times the MIC after the two-week observation period. All groups were bacteriologically negative from faecal samples taken at the end of the 10-day treatment period

The studies are different, with a different end-point in the treatment study, but demonstrate the importance of higher levels of antibiotic for treatment, presumably because the animals are clinically ill and may have depressed appetites and drug intake initially and a dilution effect from extra fluids in the diarrhoea. All samples were bacteriologically negative, however, 5 days after the start of treatment. The antimicrobial has to penetrate not just mucus layers, but also deeper into the lesions and crypts to gain access to the organism and pass through exudate, fibrin and cell debris to destroy them.

Conclusions

Relatively little is published on the pharmacokinetics of antimicrobials in the alimentary tract in comparison with the recent injectable products. Hopefully, more interest will be focused here in the future, as the bulk of antimicrobial use in pigs is via the oral route.

The small intestine is more dynamic and active than the large intestine and absorption, metabolism, excretion via the bile and breakdown in the gut all contribute to the active concentrations found there. Some products pass through relatively unchanged, others hardly reach the large intestine in an active form. There are marked differences between lincomycin and valnemulin gut concentrations, for example. The penetration of an infectious site and the drug concentration gradient required also adds another dimension, highlighted in the difference between the prevention and treatment of swine dysentery, as well as the penetration into cells in treating *L. intracellularis*.

It is interesting how well the inhibitory *in vitro* cell culture model for lawsonia conformed with the clinical study and confirmed that the intra-cellular MIC is a bio-model itself and is possibly too restrictive when set at a 99% response. It was a very good predictor of efficacy in the lincomycin case.

The pharmacodynamics of the products have an important role on the killing effect on the particular bacterial pathogens, although only time-dependent bacteriostatic antimicrobials were used as examples here. More data on bactericidal products, such as the aminoglycosides, would be useful. The relationship and interaction with other organisms in the gut have not been explored

in detail. The new resistance guidelines are causing the effects on *E. coli*, salmonella, campylobacter and enterococci to be examined.

The prevention of swine dysentery trial findings justifies that this has a valid claim, as bacterial cure is achieved. The likelihood of bacterial resistance emerging is reduced in this instance, as few, if any, bacteria are left to mutate. It cannot be argued that it is just growth promotion, using sub-inhibitory concentrations (Cyrus; personal communication). Supra-inhibitory concentrations are required to achieve the effect, although they may be lower than the treatment level for the reasons described above. Lower peri-inhibitory levels may be a cause for resistance induction concerns, as this is how resistance induction studies are carried out *in vitro*. Sub-inhibitory levels do not cause selective pressure on the bacterium to develop resistance.

To rely on treatment levels only, as once encouraged in Germany, is potentially dangerous, as clinical cure may not mean bacteriological cure. This was seen in the treatment trial and, again, peri-inhibitory levels may encourage the surviving *B. hyodysenteriae* to mutate and develop resistance mechanisms. As inclusion raises costs, farmers are keen to take products out of the feed as soon as they can, possibly before complete cure. This may explain why, in Germany, a high level of *B. hyodysenteriae* resistance to tiamulin, another pleuromutilin, is reported (Karlsson *et al*, 2002).

The need for higher levels for treatment in the second swine dysentery study highlights a common mistake made by clinicians. They use the prevention dose usually on the grounds of cost, when there is still active clinical disease present and are surprised when full control of the disease is not achieved. This is a common cause of adverse reaction reports due to lack of efficacy.

There is still much more data required to improve our understanding of gut therapy and to enable clinicians to utilize oral antimicrobials more effectively. This will enable them to make prudent, considered decisions about their use to treat patients and control antimicrobial resistance. Hopefully, this will encourage further interest in this area.

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