PHARMACOKINETIC, PHARMACODYNAMIC AND CLINICAL CORRELATIONS RELATING TO THE THERAPY OF COLONIC INFECTIONS IN THE PIG AND BREAKPOINT DETERMINATIONS

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Summary

The colonic contents concentrations (CCCs) of valnemulin, tiamulin, lincomycin, tylosin and acetylisovaleryl tylosin were related to their pharmacodynamic effect on Brachyspira hyodysenteriae and B. pilosicoli from a variety of published minimum inhibitory concentration (MIC) surveys and, where possible, these findings were correlated with clinical data to determine suitable clinical breakpoints. There were limited data available for CCC determinations and some results had to be estimated. Determinations of MICs also varied, depending on the method. The micro-broth method gave figures, on average, one dilution lower than agar plate tests; but doubling dilutions of antibiotic used in the tests also limit precision. Tests on large numbers of isolates of B. hyodysenteriae with the micro-broth test demonstrated MIC patterns where possible mutations occurred and reduced susceptibility or tolerance occurred. In the case of tylosin, there was a large jump in MICs to complete resistance development. The other antibiotics seemed to develop resistance in a more gradual stepwise way and these dips were thought to demonstrate where the effective bio-concentrations of the antibiotics reached in the colon to stimulate the mutations. When these results were compared with clinical challenge study results, with isolates of known MICs, or field experience reports, in general, a confirmatory picture evolved.

Breakpoints for the antibiotics at their normal upper usage levels were derived for brachyspiras. Valnemulin at 75ppm had an estimated breakpoint at >0.125 and >0.25µg/ml for micro-broth test and agar plate test respectively and similarly for 100ppm tiamulin >0.5 and >1.0µg/ml, lincomycin 110ppm >50 and >100µg/ml, tylosin 100ppm >16 and >32µg/ml and 100ppm acetylisovaleryltylosin >16 and >32µg/ml.

Introduction

In a previous paper, Burch *et al* (2004) demonstrated that valnemulin, a potent pleuromutilin antibiotic for the prevention and treatment of *Brachyspira*

hyodysenteriae, did not simply fit the pharmacokinetic/pharmacodynamic (PK/PD) models that were encouraged to be used in the European Guideline (EMEA/CVMP, 2001) for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances. Using the antibiotic concentration found in the colonic contents as a basic parameter, 18 times the minimum bactericidal concentration (MBC) by broth culture was required to cause complete inhibition of growth of *B. hyodysenteriae* in a prevention study and 180 times the MBC to eliminate the infection, which is the preferred endpoint in the guideline to reduce the risk of antibiotic resistance development and transfer.

It is the purpose of this paper to look further at the antibiotics tiamulin, lincomycin, tylosin and acetylisovaleryltylosin and to try to correlate their PK/PD relationships with their clinical activity, to help determine suitable clinical breakpoint relationships with their minimum inhibitory concentrations (MICs) or MBCs.

Colonic contents concentrations of various antibiotics

Colonic contents concentrations (CCCs) of various antimicrobials are not always publicly available and sometimes have to be estimated. In general terms, products that are well absorbed and metabolised in the liver tend to have low concentrations in the colon. Products that are poorly absorbed have higher concentrations, but these may also be subjected to digestive action and metabolic breakdown in the intestines. Products that are not absorbed concentrate in the large intestine and, commonly, the increase in concentration from food to faeces is 2.5 times (Burch – unpublished data).

Valnemulin and tiamulin are well absorbed (greater than 95%), extensively metabolised and excreted by the bile and their gut concentrations are relatively low (valnemulin, Econor, product data; tiamulin, Anderson *et al*, 1996). Lincomycin is moderately absorbed, metabolised in the liver and excreted by the bile and kidney and achieves moderate levels in the colon (Degeeter *et al*, 1980). Tylosin is relatively poorly absorbed (22.5% bioavailable) (EMEA, 1997). It is metabolised and excreted mainly by the liver, but the product is also broken down in the intestine to a large degree and the activity is primarily due to bio-active metabolites such as relomycin (tylosin D) and dihydrodesmycosin in the faeces. The relative 'tylosin-like' activity can be estimated at 20% of the original dose, multiplied by 2.5, to give the final colonic concentration. Acetylisovaleryltylosin has recently been registered in the European Union for enzootic pneumonia, but has been available in a number of countries, especially the Czech Republic, for the prevention and treatment of swine dysentery. There is no published data on its CCCs, although it has been extensively metabolised so that approximately 2-7% of the radio-labelled dose found in the faeces was parent compound (EMEA, 2001)

and the rest were metabolites, which may have some antimicrobial activity, but they are not specified.

Table 1 - 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.

From this data, it can be observed that there is a fairly linear relationship between the concentration given in the food and the concentration achieved in the colon contents. This means that it is difficult to set a simple breakpoint as there is often a 2.5-5-fold difference between a prevention level and a treatment level given in feed and, consequently, the antibiotic concentration achieved in the colon.

Pharmacodynamics of various antibiotics against *B. hyodysenteriae* and *B. pilosicoli*

There are a number of difficulties concerning the pharmacodynamics of the antibiotics against both *B. hyodysenteriae* and *B. pilosicoli*. There are three basic methods of growing the organisms and determining their MICs or MBCs and each one can give slightly different results, which may also affect any precision.

The three common methods for culture are broth, micro-broth and agar plates. In the broth culture, the organism is grown in doubling dilutions of antibiotic solution. The MIC is determined by the lowest concentration where the organism does not grow. The MBC is determined by sub-culturing the broth, where there is no growth into fresh broth with no antibiotic, or onto blood agar plates and seeing if the organism will grow up once the antibiotic has been diluted. The MBC is the lowest concentration of antibiotic where no growth occurs on sub-

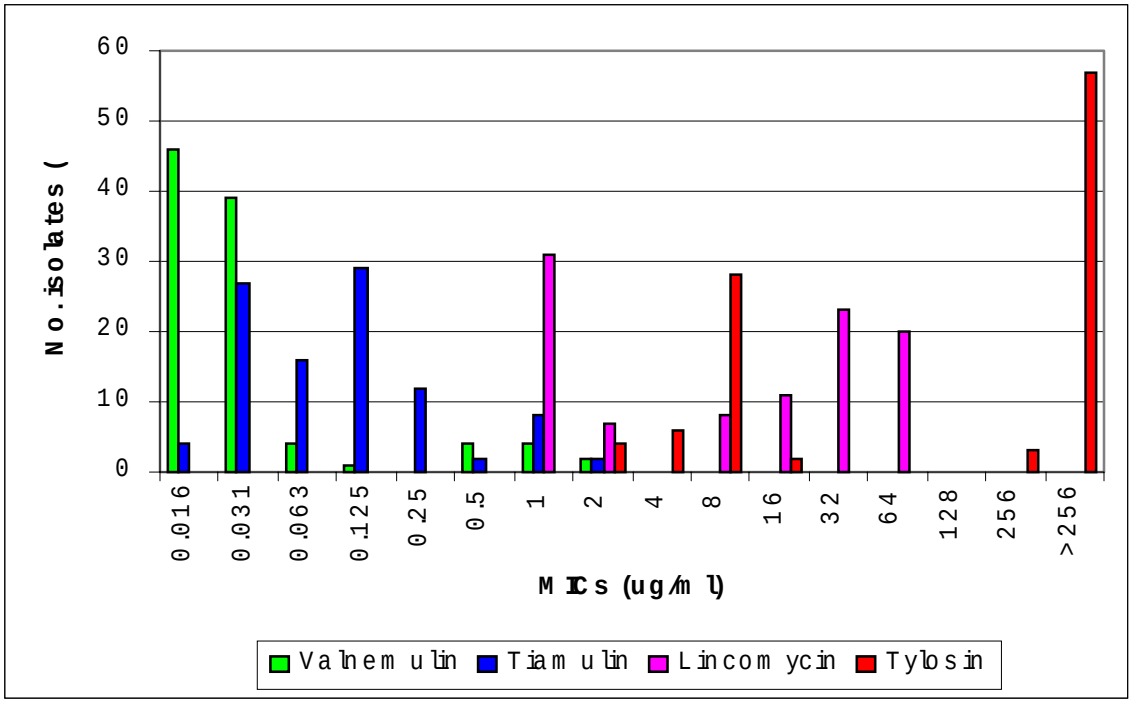
culture. Buller and Hampson (1994) demonstrated on 30 isolates of *B. hyodysenteriae* that the MBC/MIC ratio for tiamulin was, on average, 2.2 (range 1-4), lincomycin 1.7 (range 1-4) and tylosin 1.4 (range 1-4). This is a surprisingly small difference, considering the antibiotics are considered as primarily bacteriostatic. The micro-broth technique is similar to the broth technique, but uses microtitre plates. The blood agar plate test again uses doubling dilutions of the antibiotic and the plate with the lowest concentration of antibiotic, where there is no growth is the MIC. This test is probably the most widely used, especially in the UK. Interestingly, it has observed (J. R. Thomson - personal communication) that there were usually no live organisms at the site of inoculation on the agar plate test, suggesting that they have been killed and that the plate test is possibly giving an effective MBC rather than an MIC. When the MICs from micro-broth or agar plate tests were compared (Rohde *et al*, 2004) in 221 strains in Germany, it was found that there was, on average, a one dilution difference, with the micro-broth technique giving the lower result for both tiamulin (range -2 to +5) and valnemulin (range -1 to +4). This is equivalent to a MBC/MIC ratio of two, which was observed by Buller and Hampson (1994) for tiamulin.

In a very useful paper, Karlsson and others (2002) reviewed the comparative activity (MICs) of valnemulin, tiamulin, lincomycin and tylosin against 76 field isolates of *B. hyodysenteriae* in Australia, using a micro-broth double-dilution technique. It is rare to have such a large number of isolates in one report and this gives a good overview of the relative sensitivities to the antibiotics and their sensitivity patterns (see Table 2 and Graph 1).

Table 2 - *In-vitro* activity of various antibiotics against 76 Australian field isolates of *B. hyodysenteriae*

Antibiotic	MIC 50% (µg/ml)	MIC 90% (µg/ml)	Range (µg/ml)
Valnemulin	0.031	0.5	≤0.016 – 2.0
Tiamulin	0.125	1.0	≤0.016 – 2.0
Lincomycin	16	64	≤1.0 - 64
Tylosin	>256	>256	≤2.0 - >256

Graph 1 - *In-vitro* activity of various antibiotics against 76 Australian field isolates of *B. hyodysenteriae*

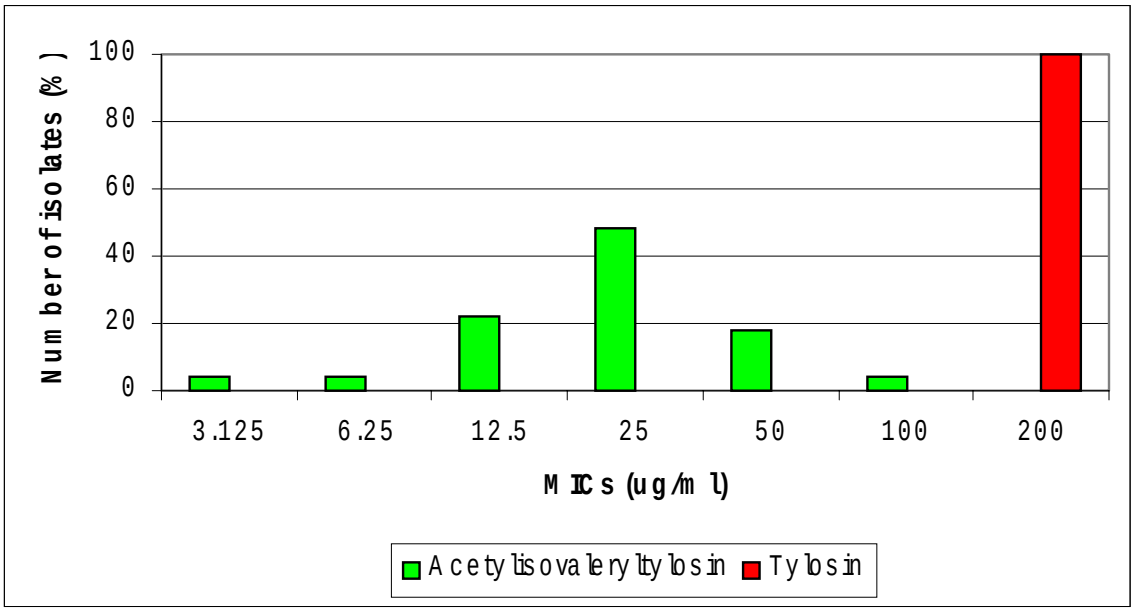


The MICs are generally lower for the pleuromutilin antibiotics in comparison with lincomycin and tylosin. If compared with the CCCs of the antibiotics, it is only the tylosin isolates that are greatly in excess eg MICs ≥ 256µg/ml in comparison with a CCC maximum of 50-100µg/g. This suggests true resistance and is reflected in the tylosin pattern of MICs, that there is a large gap between the MIC of 16 to 256µg/ml. If one looks at valnemulin by comparison, there is a bi-modal curve to the sensitivity pattern with a gap at 0.25µg/ml. Tiamulin has a tri-modal pattern with the main change between 0.5-1.0µg/ml. Lincomycin has a bimodal pattern with a gap at 4.0µg/ml and a second wave between 8-64µg/ml. This, in relation to its colon contents concentration, possibly would not identify true resistance but reduced susceptibility or increasing tolerance to the antibiotic. Karlsson *et al* (2002) postulate that at the dips, mutations, whether major or minor, have taken place in the bacteria, changing their antimicrobial susceptibility. In the case of tylosin, it is a major leap leading to frank resistance, but it is not quite so clear for the other antibiotics. The driver for these patterns is thought to be the use of antimicrobials. There is bound to be a natural range of susceptibility within a microbial population, but with products such as tiamulin, lincomycin and tylosin, which have been used in pigs for over 25 years, an antibiotic use pattern has also been sculptured and super-imposed on the natural pattern. The induction of resistance or MIC increase is characteristic for each pathogen/antimicrobial combination and the 'mutant selection window' (Drlica, 2003) is approximately around the MIC and above (to the level of the next

mutation) not usually sub-MIC. It is usually a slow and step-wise development, but sometimes there is a major jump, as in the case of tylosin. In *in-vitro* resistance development studies, the peri-MIC levels are sub-cultured and used to select for resistance. Therefore, from the above MIC study, the patterns are important to help demonstrate what are the likely active bio-concentrations achieved to have driven the mutation, at common antibiotic usage levels.

Acetylisovaleryltylosin demonstrates a clearly different sensitivity pattern from the parent compound tylosin (Cizek *et al,* 2003) using an agar plate double dilution test on 50 Czech field isolates of *B. hyodysenteriae* (see Graph 2).

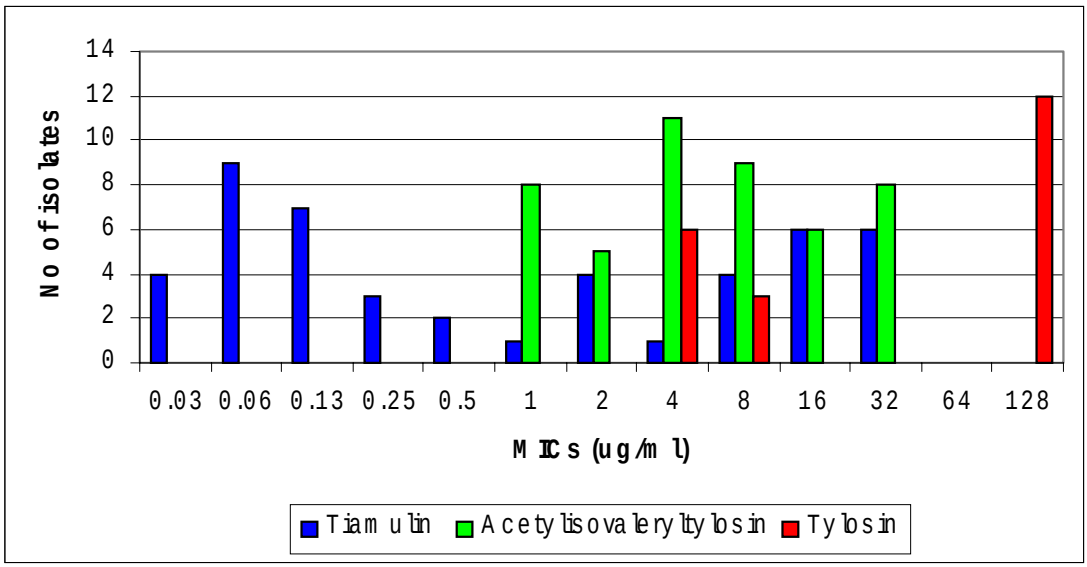
Graph 2 - Susceptibility of 50 Czech *B. hyodysenteriae* isolates to acetylisovaleryltylosin and tylosin (agar plate test)



Interestingly, they showed a uni-modal curve for acetylisovaleryl tylosin and complete resistance to tylosin. They also commented that MIC values of 12.5-25µg/ml corresponded to clinical efficacy, which would give a possible susceptibility of 78% of isolates.

Further work by Karlsson *et al* (2004) looked at the sensitivity patterns of tiamulin and acetylisovaleryltylosin against tiamulin-sensitive and resistant isolates from Sweden (24), Germany (16) and UK (10) (total – 47 isolates) and tylosin against the Swedish isolates only, using the micro-broth double dilution technique.

Graph 3 - Sensitivity/resistance patterns of 47 European isolates of *B. hyodysenteriae* for tiamulin, acetylisovaleryltylosin and tylosin



Tiamulin and tylosin showed similar resistance pattern curves, as demonstrated in Graph 1. With regard to tiamulin resistance, there is an additional wave from the MIC 4 to 32µg/ml, suggesting there may be additional mutations to achieve complete resistance. With regard to acetylisovaleryltylosin, there is an early wave followed by a second wave and then a lift at 32µg/ml, suggesting that 16µg/ml may be the cut off point for sensitivity. At the lower MICs, there did not appear to be co-resistance between acetylisovaleryltylosin and tylosin or cross-resistance with tiamulin, but at the higher levels of tiamulin resistance (mainly UK isolates), there was a definite cross-resistance to acetylisovaleryltylosin.

A similar susceptibility picture to *B. hyodysenteriae* is demonstrated against *B. pilosicoli* (Kinyon *et al*, 2002) on 25 US isolates for valnemulin, tiamulin, lincomycin and tylosin, using an agar plate test.

Table 3 - *In-vitro* activity of various antibiotics against 25 US field isolates of *B. pilosicoli*

Antibiotic	MIC 50% (µg/ml)	MIC 90% (µg/ml)	Range (µg/ml)
Valnemulin	0.06	0.5	0.03 – 2.0
Tiamulin	0.125	1.0	0.6-8.0
Lincomycin	32	64	4.0 - >128
Tylosin	>512	>512	<16 - >512

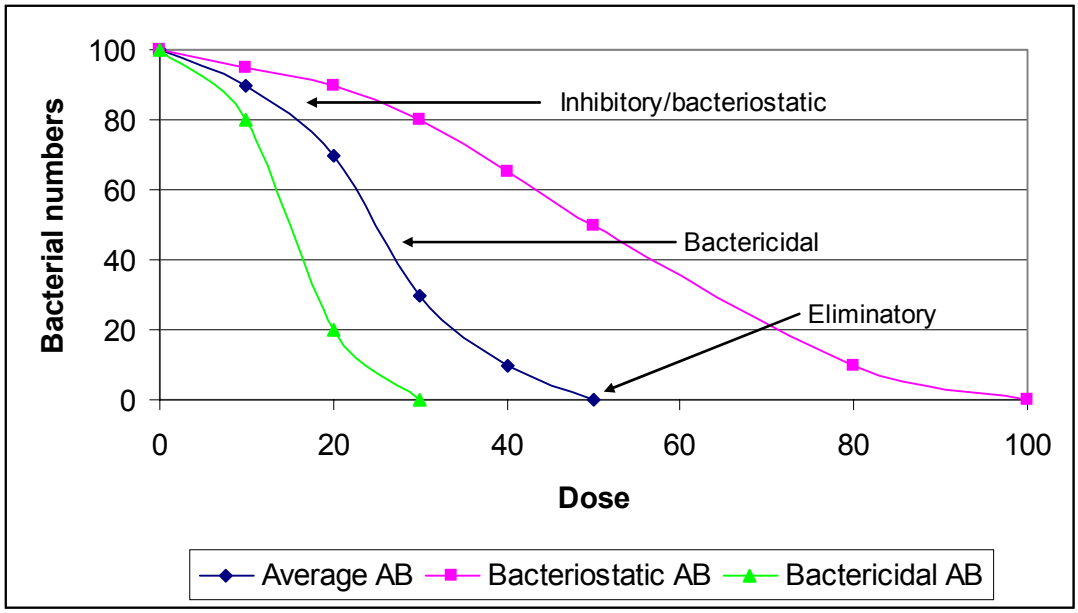
There are minor differences for the valnemulin and tiamulin levels - some isolates do exceed lincomycin's maximum CCC in the range figures and tylosin could be classed as resistant, even at the MIC50 level.

Clinical correlation of dose, effect and MIC/MBC

Dose-titration challenge studies using a known MIC/MBC of organism are the most useful for correlation purposes. For swine dysentery (*B. hyodysenteriae*) and colonic spirochaetosis 'colitis' (*B. pilosicoli*), there are two main types of study - prevention and treatment. In prevention studies, the antibiotic is administered before, at, or soon after infection, before clinical signs have appeared and some colonisation may have occurred, whereas treatment occurs when clinical signs or shedding of the organism is occurring, usually 7-21 days after infection. Normally with treatment trials, there is the treatment period of 7-21 days, followed by an observation period of 2-3 weeks, to see if the disease has been eliminated and the organism completely destroyed or whether recrudescence occurs. This is usually confirmed by autopsy and culture of the colon. In prevention studies, there are sometimes no follow-up observation periods and autopsy may be performed at the end of the trial. This may mean that the organism is not completely eliminated, but inhibited to such a level not to cause gross visible lesions, but may be present below the limit of detection or recovery by culture.

A classic dose/effect curve is represented in Graph 4. As the dose increases, initially there is an inhibitory or bacteriostatic effect, then a bactericidal effect and finally, an eliminatory effect. If an antibiotic is more bactericidal in action, the curve is steeper, if it is more bacteriostatic in action, the curve is flatter. Most bacteriostatic antibiotics do show bactericidal tendencies at very high concentrations, hence they may be used to eliminate infections, such as brachyspiras.

Graph 4 - Classic dose/effect curves for antimicrobial substances



Valnemulin

In Burch *et al* (2004), 10ppm valnemulin inhibited the growth of the isolate of *B. hyodysenteriae* and prevented lesions and clinical disease, but there was no observation period, so one could not say absolutely that it had eliminated the organism. In fact, in the same study, pigs on higher levels of valnemulin broke down with dysentery when afflicted with a concurrent disease problem and they had stopped eating. One can only assume that it is having a bacteriostatic effect and not a bactericidal/eliminatory effect. In the treatment trial, there was a 14-day observation period and although there was a good clinical response (bactericidal effect) at 75ppm valnemulin (the recommended use level), the organism was only eliminated at 100ppm.

Table 4 -Correlation of clinical effect of valnemulin with CCC and MIC/MBC determined by broth and agar methods

		Effective bio-concentration at site of infection based on different MIC/MBC determinations / CCC/MIC MBC ratios		
Valnemulin	CCC (µg/ml)	MIC broth	MBC broth	MIC/MBC agar
10 ppm (bacteriostatic/inhibitory)	0.24	0.003	0.0125	0.025
CCC/MIC ratio		80	19	10
100 ppm (10 x 10ppm) (bacteriostatic/inhibitory)	2.4	0.03	0.125	0.25
100 ppm (eliminatory)	2.4	0.003	0.0125	0.025
CCC/MIC ratio		800	192	96

From these calculations, the effective bio-concentration at the site of infection is not the same as the CCC for valnemulin. Substantially higher CCCs are required to inhibit and eliminate *B. hyodysenteriae*. Of interest, the effective bio-concentration at 100ppm in the feed in relation to the MBC broth and the MIC/MBC agar is of the same order as the first dip in the sensitivity pattern, demonstrated in Graph 1, of 0.125-0.25µg/ml. The product can be used up to 200ppm, which would give an effective inhibitory bio-concentration of 0.25-0.5µg/ml.

Tiamulin

Taylor (1980) described a dose-titration challenge study for the prevention of swine dysentery. The MIC of the isolate of *B. hyodysenteriae* (isolate S73/2) used was determined by the broth method at 0.05µg/ml (Taylor, 1976). The pigs were given tiamulin medicated feed (0, 25, 30, 35 and 40ppm) from 5 days before infection for 42 days post-infection. The pigs were infected on day 0 and 1. Autopsies were carried out following a 21-day observation period on unmedicated food. No dysentery occurred during the trial and *B. hyodysenteriae* was not recovered from the faeces during or after treatment. However, on autopsy, it was recovered from colon scrapings in 2/5 pigs in groups given 35 and 40ppm tiamulin.

In a second study, using tiamulin in the drinking water at 22.5, 45 and 60ppm for 3-5 days for the treatment of swine dysentery, pigs were challenged on 3 occasions with the same *B. hyodysenteriae* isolate as above and treatment started when clinical signs developed. They were autopsied 21 days after the end of

treatment, giving time for the disease to develop. Only the tiamulin at 60ppm in water groups completely eliminated the infection. Dysentery returned in the 45 and 22.5 ppm treated groups and *B. hyodysenteriae* were re-isolated in the observation period, colonic lesions were observed and *B. hyodysenteriae* were isolated at autopsy.

In a third study, pigs were infected with a different isolate of *B. hyodysenteriae* (isolate S80/5) with an MIC of 0.3-0.5µg/ml by a broth method (Taylor, 1982). The pigs were challenged three times and treated when clinical signs of dysentery developed. Tiamulin was given at 0, 50, 80, 120 and 160ppm for 14 days in the feed and the pigs autopsied 21 days after the end of treatment. Pigs in treatment groups 120 and 160 ppm responded quickly to treatment and *B. hyodysenteriae* were not isolated in the treatment or observation period, but were from the majority of pigs at autopsy. These levels showed a clear bactericidal activity but not an eliminatory effect. In contrast, the 50 and 80ppm groups showed some clinical signs throughout the trial and also *B. hyodysenteriae* could be isolated. At autopsy, *B. hyodysenteriae* could be isolated from almost all of the pigs. An inhibitory and low level bactericidal effect was seen at these levels.

Table 5 - Correlation of clinical effect of tiamulin with CCC and MIC determined by a broth method

Tiamulin Trial 1.	**CCC (µg/g)**	**Effective bio-concentrations at site of infection / CCC/MIC ratios**
25ppm (feed) (bacteriostatic/inhibitory)	0.78	MIC 0.05µg/ml (S73/2)
CCC/MIC ratio		16
Trial 2.		
22.5ppm	0.81	
CCC/MIC ratio		16
45ppm (water) (bactericidal)	1.62	
CCC/MIC ratio		32
60ppm (water) (eliminatory)	2.16	
CCC/MIC ratio		43
Trial 3.		MIC 0.5µg/ml (>0.25µg/ml) (S80/5)
50ppm (feed) (inhibitory/bactericidal)	1.51	
CCC/MIC ratio		3 (6)
80ppm (feed) (inhibitory/bactericidal)	2.49	
CCC/MIC ratio		5 (10)
120ppm (feed) (bactericidal)	3.74	
CCC/MIC ratio		8 (15)
160ppm (feed) (bactericidal)	4.98	
CCC/MIC ratio		10 (20)

The correlations lack precision between the two studies but, in the first and second trial, it takes at least 15 times the CCC to achieve an inhibitory affect with a very sensitive strain. This has not been titrated down further than 25ppm so may go lower, but as the concentration increases, a bactericidal effect and eliminatory effect are seen when tiamulin was given in drinking water. With the higher MIC isolate in trial 3, Taylor (1982) suggests the MIC is above 0.25µg/ml and may be below 0.5µg/ml, but it is difficult to be more precise with doubling dilutions. Both figures are used in Table 5 for comparison. Using the CCC/MIC ratios to predict effect from trial 1 and 2, an eliminatory effect might be achieved with the more sensitive strain (S73/2) at 75ppm tiamulin and above in the feed, but may not be reached with the less sensitive isolate (S80/5) until potentially 320-640ppm if the correlations are correct. Tiamulin is approved upto 200 ppm in Australia, so could be eliminatory in many susceptible cases, but may only be inhibitory at MICs of 1.2-2.0µg/ml. The lowest point of the sensitivity pattern, just before the next wave, is 0.5µg/ml (see Graph 1) which tends to confirm these findings.

Lincomycin

Cowan and Duhamel (1997) described a dose-titration study for the treatment of colonic spirochaetosis caused by an artificial challenge with *B. pilosicoli* with a reported MIC of 50µg/ml using an agar plate method (J. Kinyon, personal communication). With doubling dilutions, it is greater than 25µg/ml, but may not be as high as 50µg/ml. Two weeks post-infection, the pigs were treated with feed containing 0, 22, 33 and 110ppm of lincomycin for three weeks and there was a two-week observation period. Autopsies on the pigs were not performed. There were clinical improvements in the 33 and 110ppm groups and *B. pilosicoli* shedding stopped in these groups in the first week of treatment and for the rest of the study. In the 22ppm group, diarrhoea continued throughout the study and *B. pilosicoli* was isolated each week and had no disease control effect.

Table 6 - Correlation of clinical effect of lincomycin with CCC and MIC determined by a broth method

Lincomycin	**CCC (µg/g)**	**Effective bio-concentrations at site of infection**
22ppm (feed) (no effect)	8.5	MIC 50µg/ml (>25µg/ml)
CCC/MIC ratio		0.17 (0.34)
33ppm (feed) bactericidal	12.7	
CCC/MIC ratio		0.25 (0.51)
110ppm (feed) bactericidal	38.2	
CCC/MIC ratio		0.76 (1.53)

These results are in complete contrast with earlier findings with the pleuromutilins, where much higher CCCs were found in comparison with the MICs. Even using the MIC at >25µg/ml, the CCC does not become exceeded until 110ppm, and it is difficult to explain how the 33ppm worked so well. It would suggest that either the MIC figure is high, or the colon concentrations are too low, or another action is taking place. The MIC was determined by agar plate test, which is commonly higher than the broth tests by one dilution (Rohde *et al,* 2004) so could be between 25 and >12.5µg/ml. The DeGeeter *et al* (1980) paper also has a possible discrepancy between the comparative ileal and colon contents concentrations between the two dose rates 110 and 220ppm and may have lowered the average CCC figure, which should be approximately 15µg/g. As both *B. hyodysenteriae* and *B. pilosicoli* are primarily surface dwelling organisms, it is not thought that other factors, such as higher intracellular concentrations, whether epithelial or macrophage, influence the figures. Once the adjusted MIC and pharmacokinetic factors are tailored into the equation, the equivalent CCC/MIC ratio falls between 0.6 and 1.2, suggesting the effective inhibitory concentration at 110ppm lincomycin could reach 50µg/ml and at the maximum recommended

usage level, 220ppm, 100µg/ml by micro-broth test. This could account for the shifting resistance trend pattern seen in Graph 1. *B. pilosicoli* may be more easily controlled as the inflammatory reaction is less severe and less protein-rich inflammatory debris (including blood) is produced.

From the author's personal field experience (unpublished data) *B. hyodysenteriae,* with a lincomycin MIC 16µg/ml derived from agar plate tests, can be treated (inhibitory, bactericidal effect but not eliminatory) by 44ppm lincomycin in the food and 33ppm lincomycin in the drinking water (as part of a combination with spectinomycin) and *B. pilosicoli*, with an MIC of 4µg/ml, eliminated with 33ppm lincomycin in the water (equivalent to 66ppm lincomycin in the feed). This suggests that the effective inhibitory bio-concentration in the colon is closer to the actual CCC (CCC/MIC ratio of 1) in contrast to the pleuromutilins. One additional factor may be the comparatively low protein binding of lincomycin at 4% in plasma reported in EMEA (1998) unlike tylosin and tiamulin, which are reported at 30 and 50% respectively (Ziv, 1980) in chickens. With reference to Graph 1, it would suggest that the first wave is of highly sensitive organisms and the second wave may still be all susceptible to an inhibitory effect, depending on the level of lincomycin included in the feed. There would appear to be a number of high MIC isolates of *B. pilosicoli*, >128µg/ml (Kinyon *et al,* 2002), which might suggest actual resistance.

Tylosin

There is no recent dose-titration study data available for tylosin to make any determinations. It is well recognised that the majority of isolates of *B. hyodysenteriae* and *B. pilosicoli* are resistant to tylosin. From Graph 1, there is a clear jump from the first wave to the second. This suggests that organisms with an MIC of upto 16µg/ml may actually be susceptible, but there has been a mutational jump from there to the128µg/ml resistant isolates. It can be surmised that the bio-effective concentrations achieved in the colon are in the order of 16µg/ml when administered at 100ppm tylosin in the feed and this has driven the resistance mutation step. A CCC/MIC ratio of 3.1 could be considered inhibitory.

Acetylisovaleryltylosin

Acetylisovaleryltylosin has recently been reported for the prevention (50ppm) and treatment (100ppm) of swine dysentery (Tasker *et al*, 2004) but unfortunately there are no dose-titration or challenge strain MIC results publicly available. Cizek *et al* (2003) reported that clinical efficacy was seen against isolates with MICs of 12.5-25µg/ml by agar plate test, so could be lower by one dilution by broth test at 6.3 and 12.5µg/ml and this correlates quite well with the dip in Graph 3 at 16µg/ml. This also correlates quite well with the average CCC

estimated from the radio-labeled studies of 5.5µg/g for 50ppm and 11.3µg/g for 100ppm (upper ranges 8.8-17.5µg/g, respectively) giving a CCC/MIC ratio of about one. It does not include any activity associated with microbiologically active metabolites, so the effective CCC may be higher.

Conclusions and Discussion

The data cannot be considered complete and there are still many variations in studies, methods of MIC determinations, doubling dilutions etc., so absolute precision cannot be completely achieved. However, a pattern appears to be developing, which may help the clinician determine the clinical breakpoint for a particular isolate, against a particular antibiotic by a particular method and select an effective dose rate.

The estimated inhibitory breakpoints are summarised in Table 7. They do not reflect bactericidal or eliminatory levels that might be achieved with the in-feed inclusion levels represented.

Table 7 - Clinical inhibitory breakpoints (MICs) for brachyspiras by both micro-broth and agar plate methods (Agar plate tests approximately double broth and micro-broth tests)

Antibiotic (level in feed ppm)	Micro-broth test MIC breakpoint (µg/ml)	Agar plate test MIC breakpoint (µg/ml)
Valnemulin (75)	>0.125	>0.25
Tiamulin (100 UK) Tiamulin (200 AUS, 220 US)	>0.5 >1.0	>1.0 >2.0
Lincomycin (110)	>50	>100
Tylosin (100)	>16	>32
Acetylisovaleryltylosin (100)	>16	>32

Rønne and Szancer (1990) previously reported their estimations for breakpoints using agar plate tests only (see Table 8).

Table 8 - Estimations for MIC breakpoints for *B. hyodysenteriae* using agar plate tests in Denmark (Rønne and Szancer, 1990)

	MIC breakpoints (µg/ml)		
Antibiotic	Sensitive	Intermediate	Resistant
Tiamulin	≤1.0	>1.0 to ≤4.0	>4.0
Lincomycin	≤4.0	>4.0 to ≤36	>36
Tylosin	≤1.0	>1 to ≤4	>4

The breakpoints for tiamulin are relatively similar to the Rønne and Szancer (1990) results. With regard to lincomycin, the breakpoint is approximately 3 times higher in this study, but their breakpoint was based on the DeGeeter *et al* (1980) data as well, which may have an underestimation of CCC at the 110ppm level. The tylosin data by contrast relates to colon tissue, rather than colon contents, and would appear to be much lower than the present study, which takes into account CCC and MIC patterns, although there is a dearth of good clinical data.

As more data becomes available, clearer interpretations may be made. It is a difficult area to work in, as there are so many variables to take into account. However, as the methods become more standardised and more information on gut pharmacokinetics becomes available, which can be correlated with clinical work, more precise determinations can be made.

References

Anderson, M.D., Stroh, S.L. and Rogers, S. (1996). Proceedings of the American Association of Swine Practitioners, Chicago, Illinois, USA, pp. 118-121.

Buller, N.B. and Hampson, D.J. (1994). Australian Veterinary Journal, **71**, 211-214.

Burch, D.G.S., Ripley, P.H. and Burrows, M. (2004). The Pig Journal, **54**, 198-205.

Cizek, A, Lobova, D. and Smola, J. (2003). Second Colonic Spirochaetal Infections in Animals and Humans Conference, Eddleston, Scotland, Abstract 55.

Cowan, T.R. and Duhamel, G. (1997). Proceedings of the American Association of Swine Practitioners, Quebec City, Canada, pp. 225-229.

DeGeeter, M. J., Barbiers, A.R. and Stahl, G.L. (1980). Proceedings 6th International Pig Veterinary Society Congress, Copenhagen, Denmark, p. 283.

Drlica, K. (2003). Journal of Antimicrobial Chemotherapy, **52**, 11-17.

EMEA (1997). EMEA/MRL/205/97-Final 'Committee for Veterinary Medicinal Products – Tylosin – Summary Report 3.'

EMEA (1998). EMEA/MRL/497/98-Final 'Committee for Veterinary Medicinal Products – Lincomycin – Summary Report 1.'
EMEA (2001). EMEA/MRL/794/01-Final 'Committee for Veterinary Medicinal Products – Acetylisovaleryltylosin – Summary Report 2.'
EMEA/CVMP (2001). EMEA/CVMP/627/01-Final 'Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances.'
Karlsson, M., Oxberry, S.L. and Hampson, J. (2002). Veterinary Microbiology, **84**, 123-133
Karlsson, M., Aspan, A., Landen, A. and Franklin, A. (2004). Journal of Veterinary Microbiology, **53**, 281-285
Kinyon, J.M., Murphy, D. Stryker, C., Turner, V., Holck, J.T. and Duhamel, G. (2002). Proceedings 17th International Pig Veterinary Society Congress, Ames, Iowa, USA, 2, p. 50
Rohde, J., Kessler, M., Baums, C.G. and Amtsberg, G. (2004). Veterinary Microbiology, **102**, 25-32.
Rønne, H. and Szancer, J. (1990). Proceedings 11th International Pig Veterinary Society Congress, Lausanne, Switzerland, p. 126.
Tasker, J.B., Burrows, M.R. and Kavanagh, N.T. (2004). Proceedings 18th International Pig Veterinary Society Congress, Hamburg, Germany, 1, p. 313.
Taylor, D.J. (1976) Proceedings 4th International Pig Veterinary Society Congress, Ames, Iowa, USA, p. L25.
Taylor, D.J. (1980). Veterinary Record, **106**, 526-528.
Taylor, D.J. (1982). Proceedings 7th International Pig Veterinary Society Congress, Mexico City, Mexico, p. 47.
Ziv, G. (1980). Veterinary Quarterly, **2**, 4, 206-210