Managing *Lawsonia* and *Brachyspira* infections using pharmacokinetic and pharmacodynamic principles

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Introduction

To make informed decisions on which antibiotic to use and which route and at what inclusion/dosage level, it is important for practitioners to be familiar with the basic pharmacokinetic (PK) information for a drug and be able to relate it to the pharmacodynamic (PD) information that is available. Frequently, the minimum inhibitory concentration (MIC) is determined for *Brachyspira hyodysenteriae*, the cause of swine dysentery, as part of the diagnostic work up, as the usual PD information and this can be correlated with the PK or concentrations of antibiotic found in the colon contents. Similarly, for *Lawsonia intracellularis* infections, the cause of porcine proliferative enteropathy ('ileitis'), the ileum contents concentration is the major PK information but it is very difficult to grow *L. intracellularis* in cell cultures, so there is generally very limited intracellular MIC (iMIC) data available from field investigations.

The purpose of this paper is to correlate the PK and PD information for tiamulin (Denagard – Novartis Animal Health Inc.) against these two infections, so that the practitioner can utilise his field information to make more informed treatment decisions regarding these primary enteric pathogens.

Pharmacokinetics of tiamulin in the gut

The concentrations that were achieved in the colon contents,[1] using a microbiological method, following administration of tiamulin in the feed at 38.5, 110 and 220 ppm for 14 days are summarised in Table 1. It also looked at tiamulin's colon contents concentration (CCC) after giving it in the drinking water at 60, 120 and 180 ppm for 5 days (see Table 1). Drugs accumulate in the colon contents and that the ileum contents concentration (ICC) was shown to be approximately 29% of the CCC.[3]

These concentrations are helpful in giving an idea of the tiamulin-like bioactive concentrations that can be achieved in the certain sections of the gut. There may be some binding of the drug to the gut contents but this has not been taken into account. Tiamulin is only a moderate serum-protein binder in pigs at about 30%. Dose for dose, in feed medication achieved higher concentrations in the gut than water medication, possibly due to feed impairing the absorption and bioavailability of tiamulin.

Pharmacodynamics

Lawsonia intracellularis

Recent pharmacodynamic data for *L. intracellularis*[1,2] really pushed forward our knowledge and understanding of how drugs worked against this infectious agent. Early pioneering work[6,7] gave us some indication of what antibiotics were likely to work against *L. intracellularis*, but they used a limited number of isolates and limited drug concentrations. A wider drug concentration range[1,2] was used to test 10 US and EU isolates, to determine their iMICs and extracellular MICs (eMICs) and the determination was repeated (see Table 2). It was demonstrated[3] that the iMICs were probably more significant when it came to determining PK/PD relationships, as *L. intracellularis* penetrates epithelial cells rapidly, within hours, leaving little time for exposure to an antibiotic and the iMIC determination more simulated the exposure to infected epithelial cells in the gut by antibiotics given in the feed and water.

In addition different susceptibility patterns could be demonstrated for different antibiotics, which suggested that reduced susceptibility or even resistance development may have evolved in some cases, e.g. tetracycline and lincomycin but not to tiamulin, valnemulin, carbadox and tylosin.

Brachyspira hyodysenteriae

Interest in *B. hyodysenteriae* waned in the US over the last decade but has recently been rekindled with a number of outbreaks of *Brachyspira* spp associated diarrhoeas[4] in N. America. Europe has been struggling with the disease and resistance issues, especially since 2006 when certain growth promoters were banned, which also inhibited *B. hyodysenteriae*.

One of the first major reports[5] described the MICs of 76 Australian isolates of *B. hyodysenteriae*, using a new microbroth dilution method (see Table 3).

From this data, sufficient information was available to formulate susceptibility patterns for the different antibiotics (see Figure 2).

Table 1: Tiamulin colon and ileum (estimated) contents concentration following administration via feed and drinking water.

Tiamulin concentration (ppm)	Dosage rate (mg/kg bwt)	Colon contents concentration (µg/ml)	Ileum contents concentration (µg/ml) (Estimated ICC = CCC × 29%)
In feed (14 days)			
38.5	1.98	< 1.98 (LOQ) 0.99 (E)	0.29
110	6.6	2.84	0.82
220	13.2	8.05	2.33
In water (5 days)			
60	6.16	2.16	0.63
120	13.2	5.59	1.62
180	20.9	18.58	5.39

Table 2: *L. intracellularis* intracellular and extracellular MIC 50, MIC 90 and range (2 × 10 isolates).

Antimicrobial	iMIC50 (µg/ml)	iMIC 90 (µg/ml)	iMIC Range (µg/ml)
Tiamulin	0.125	0.125	0.125 - 0.5
Valnemulin	0.125	0.125	0.125
Tylosin	2.0	8.0	0.25 - 32
Lincomycin	64	>128	8.0 - >128
Chlortetracycline	8.0	64	0.125 - 64
Carbadox	0.125	0.25	0.125 - 0.25
Antimicrobial	**eMIC50 (µg/ml)**	**eMIC 90 (µg/ml)**	**eMIC Range (µg/ml)**
Tiamulin	4.0	8.0	1.0 – 32
Valnemulin	0.25	1.0	0.125 – 4.0
Tylosin	16	128	1.0 - >128
Lincomycin	>128	> 128	32 - >128
Chlortetracycline	64	64	16 – 64
Carbadox	4.0	16	1.0-32

Table 3: *B. hyodysenteriae* MIC 50, MIC 90 and range against a number of antibiotics.

Antibiotic	MIC 50 (µg/ml)	MIC 90 (µg/ml)	MIC range (µg/ml)
Tiamulin	0.125	1.0	≤ 0.016-2.0
Valnemulin	0.031	0.5	≤ 0.016-2.0
Tylosin	> 256	>256	8.0->256
Lincomycin	16	64	≤1.0-64

Tylosin showed a single-step resistance pattern, with 16 μg/ml being the 'wild type' cut off or breakpoint, whereas tiamulin shows a 2-step mutation pattern normally, with a 'wild type' cut off at 0.5μ/ml and then a first stage mutation to 2.0 μg ml. In Europe we have found strains with MICs of > 4.0μg/ml (a second mutation), which is considered the resistance breakpoint for tiamulin.

Figure 1: Comparative susceptibility pattern of tiamulin and lincomycin iMICs against *L. intracellularis.*

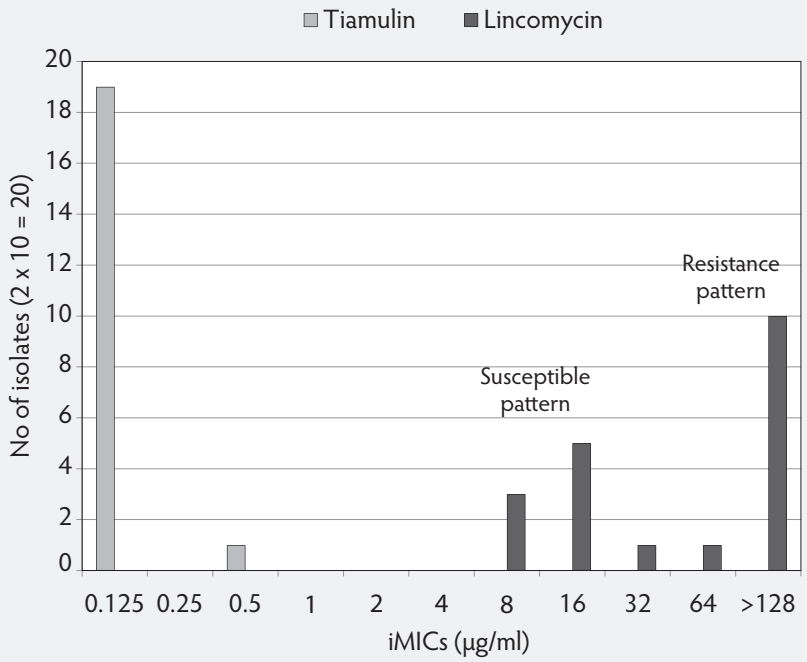
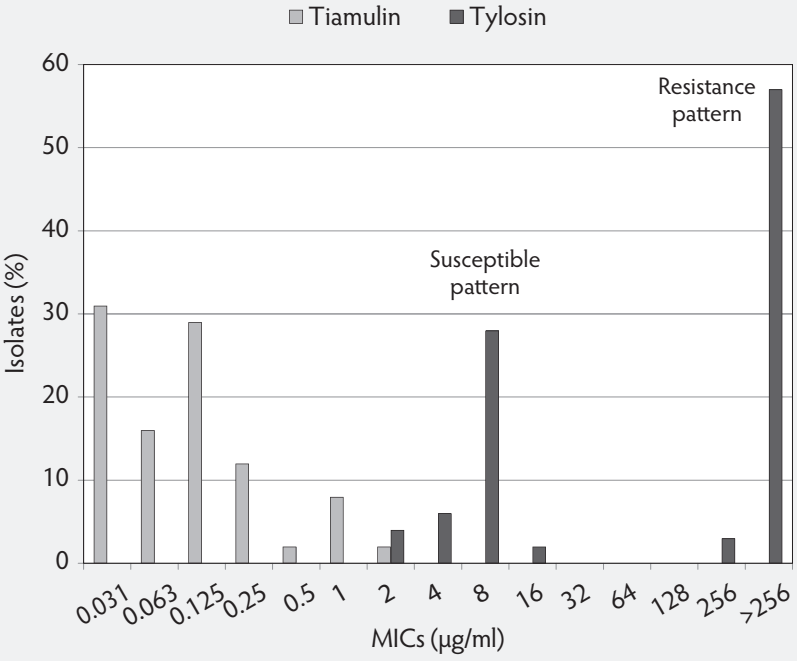


Figure2: Comparative susceptibility patterns for tiamulin and tylosin against *B. hyodysenteriae.*



Integrating PK with PD

Lawsonia intracellularis

Using a simple approach the concentration of the drug should be above the MIC of the organism for sufficient time to either inhibit its growth or to kill the organism. The effect the drug has on the bug is dependent on the type of antibiotic, whether it is bacteriostatic or bactericidal and the organism itself. In the case of *L. intracellularis* the drug needs also to penetrate the cell to exert its antibiotic effect.

Tiamulin at 38.5ppm would be expected to exert an inhibitory effect (see Figure 3) against the majority of *L. intracellularis* isolates. The iMIC and intracellular minimum bactericidal concentration (iMBC) have not been determined so the iMBC: iMIC ratio has not been established. However, a trial[11] showed there was an inhibitory effect when given in the feed at 38.5ppm for 28 days to pigs already infected with *L. intracellularis*, with an MIC of 0.25μg/ml. Earlier work[8] showed that 50ppm tiamulin in feed given prior to infection completely prevented the development of lesions caused by a strain of *L. intracellularis* with an iMIC of 0.125μg/ml. Tiamulin at 150ppm administered to pigs 7 days after infection for 14 days completely eliminated the infection, demonstrating a more bactericidal, eliminatory effect[8] at ICC concentrations 13 times the iMIC.

Brachyspira hyodysenteriae

With regard to *B. hyodysenteriae*, the MIC susceptibility pattern can be compared with the CCCs achieved with tiamulin given in feed and in water (see Figure 4).

Tiamulin at 38.5ppm in feed can be expected to be inhibitory to most of the 'wild type' isolates up to 0.5 μg/ml. A trial[9] demonstrated a complete inhibitory effect at 25-40 ppm tiamulin in

feed against an isolate of *B. hyodysenteriae* with an MIC of 0.05 µg/ml. At tiamulin 110 ppm in feed, a bactericidal effect against 'wild type' isolates might be expected as the MBC: MIC ratio is approximately 2: 1[2] and an inhibitory effect against the first-step mutants. A similar effect could be expected with water medication at 60 ppm and a strong bactericidal, eliminatory effect with tiamulin at 60 ppm in the water for 3-5 days against an isolate with an MIC of 0.05 µg/ml[9] was shown and at 100ppm and above in feed for 7-14 days against an isolate with an MIC of 0.5 µg/ml.[10] Concentrations of tiamulin in the colon following 220 ppm would expect to achieve a bactericidal effect against both wild type and first-step mutants and could be considered to be achieving a mutant prevention concentration, which should limit resistance development.

Conclusions

An understanding of the PK and PD relationships can help assist the practitioner to decide, which drug to use and at what concentration/dose to achieve the optimum results regarding treatment and in the case of swine dysentery, even eradication. It is just possible, with the use of mutant prevention concentrations of tiamulin, that resistance development can be slowed or even halted.

Regarding ileitis, it would appear that some isolates are demonstrating reduced susceptibility even possible resistance to some antibiotics, so again care in selection of the right antibiotic, at the right dose to get a good and quick response is increasingly important.

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Figure 3: Concentration of tiamulin in the ileum contents in relation to the iMIC susceptibility pattern of *L. intracellularis.*

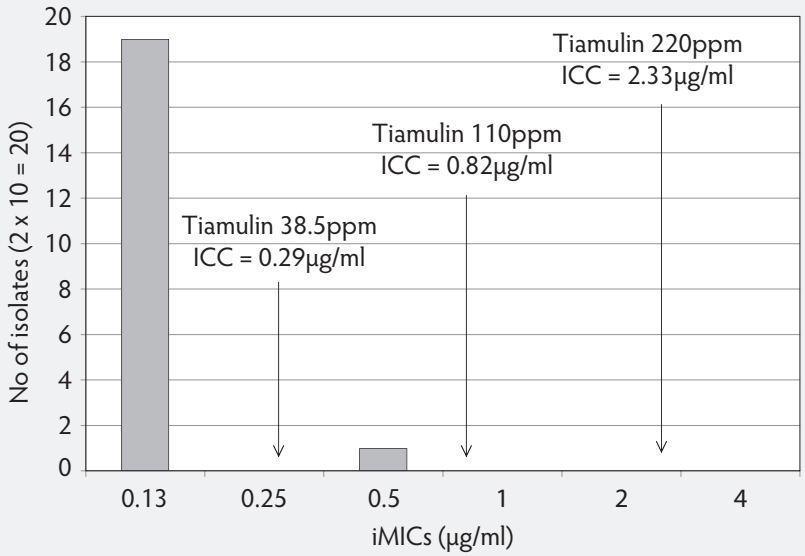
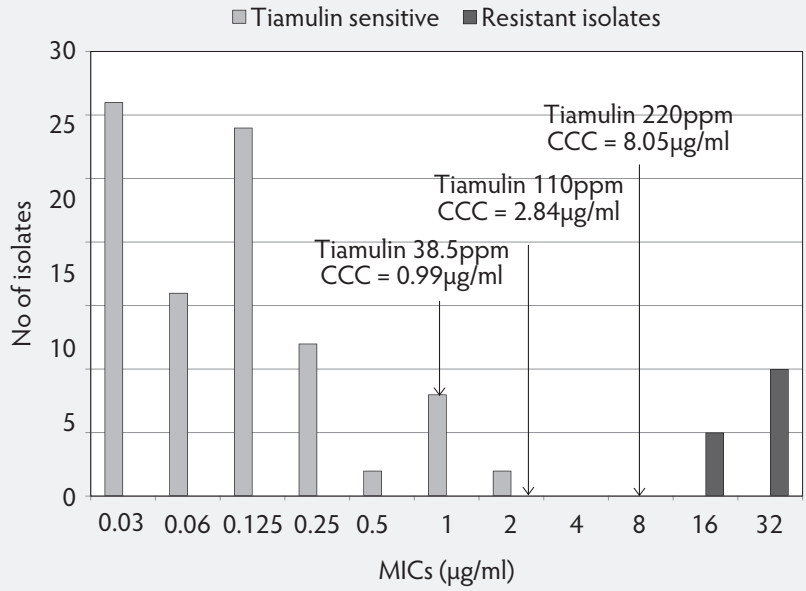


Figure 4: Concentration of tiamulin in the colon contents in relation to the MIC susceptibility pattern of *B. hyodysenteriae.*



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