Papers on Tiamutin presented at the
18th International Pig Veterinary Society Congress
Hamburg, Germany – 27 June-1 July 2004

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INTRODUCTION

As a world-leading animal health company, Novartis, manufacturer of TIAMUTIN®, is proud to be a sponsor of the 18th IPVS Congress – the source of so much significant new scientific research material.

For over 25 years TIAMUTIN, the original member of the pleuromutilin family of antibiotics, has evolved as a leading therapeutic product for "pneumo-enteric" diseases.

TIAMUTIN is highly effective against traditional pig diseases like enzootic pneumonia and swine dysentery, yet it is also a valuable weapon against emergent diseases such as ileitis and mycoplasmal arthritis. Synergistic activity, when combined with chlortetracycline (CTC) or doxycycline, makes it especially suitable for broader spectrum control of mixed infections. As a long-proven aid for the pig industry TIAMUTIN can definitely be considered "the reliable productivity protector".

TIAMUTIN offers a flexible range of formulations for feed, water and injection and has been developed, manufactured and marketed exclusively for animal medicine. It is not related to or identical to human-use medicinal antibiotics, unlike tylosin and lincomycin, and does not select for cross-resistance or co-resistance to them.

Given today's increasing concerns regarding antibiotic use, this makes TIAMUTIN an ideal choice for disease therapy and control and, like ECONOR®, a prudent choice for today's progressive pig producers.

It is our hope that these Proceedings – a collection of papers presented on TIAMUTIN at the 18th IPVS – will be a reminder of Novartis Animal Health's commitment to the global pig industry and will assist you in your production-related decisions.

Dr. Clive Girdler

Global Category Manager – Pig Products

Dr. Ulrich Klein

International Technical Services Manager – Pig Products

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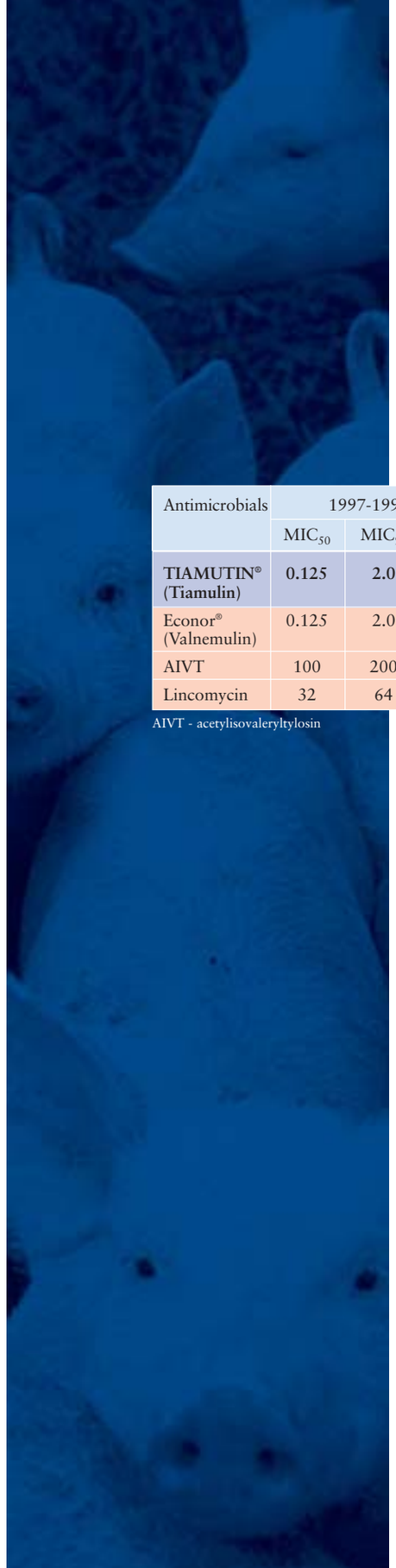

A. Cizek and others

In vitro susceptibility to four antimicrobials in Czech isolates of *Brachyspira pilosicoli*

The antimicrobial susceptibility of *B. pilosicoli* strains isolated in the Czech Republic between 1997-1999 and 2001-2003 to four antimicrobials was compared and the trend of the development of reduced sensitivity was evaluated.

Table 1. MICs (µg/ml) of 33 *B. pilosicoli* strains isolated in Czech Republic between 1997-1999 and 2001-2003

Antimicrobials	1997-1999 (n = 18)			2001-2003 (n = 15)			Total (n = 33)		
	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range
TIAMUTIN® (Tiamulin)	**0.125**	**2.0**	**0.03- 8.0**	**0.250**	**1.0**	**0.03-1.0**	**0.125**	**1.0**	**0.03- 8.0**
Econor® (Valnemulin)	0.125	2.0	0.03-4.0	0.125	1.0	0.03-2.0	0.125	2.0	0.03-4.0
AIVT	100	200	3.125-200	50	200	3.125-200	50	200	3.125-200
Lincomycin	32	64	0.5-128	2.0	32	0.5-128	16	64	0.5-128

AIVT - acetylisovaleryltylosin

Key facts

- *Low tiamulin and valnemulin MIC values were found during the evaluated time periods in comparison to AIVT and lincomycin.*
- *The results reveal no trend towards increasing MICs for both pleuromutilins.*
- *MIC values of tiamulin and valnemulin isolated between 2001-2003 remained practically unchanged in comparison to those four years ago.*
- *The data confirm the low sensitivity of B. pilosocoli to both AIVT and lincomycin.*
- *Tiamulin and valnemulin are the antibiotics of choice for the treatment of Porcine Colonic Spirochaetosis (PCS) in the Czech Republic.*

In vitro susceptibility to four antimicrobials in Czech isolates of *Brachyspira pilosicoli*

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Introduction and Objectives

Brachyspira pilosicoli is the causative agent of porcine intestinal spirochaetosis (PIS), which is manifested by mild colitis and decreased growth rates of growing pigs[7]. This endemic disease was earlier proved in many countries with major swine production. Antimicrobial drugs effective against *B. pilosicoli* cover a spectrum similar to that of *B. hyodysenteriae*.

The aim of this study was to evaluate MICs of pleuromutilins (tiamulin, valnemulin), acetylisovaleryl-tylosin (AIVT) and lincomycin using a set of randomly-selected isolates of *B. pilosicoli* obtained from pig farms in the Czech Republic between 1996 and 2003.

Material and Methods

The study included a total of 33 *B. pilosicoli* isolates which were obtained from 29 pig farms in the periods of 1996-99 and 2001-03. Sixteen (55%) pig farms were with swine dysentery history. The only restriction was that no more than two isolates from individual farms from the period under investigation were selected.

B.pilosicoli isolates were confirmed by testing of biochemical activity[3] and PCR-RFLP[6]. The type strains of *B. hyodysenteriae* B78^T (ATCC 27164^T), B. innocens B256^T (ATCC 29796^T), *B. pilosicoli* P43/6/78 (ATCC 51139^T) and *B. murdochii* 56-150^T (ATCC 51284^T) were used for identification processes testing.

The Wilkins-Chalgren anaerobe agar (CM 619, Oxoid) with 5% ovine blood (WCABA) was used to determine MICs of the antimicrobials tiamulin, valnemulin (Novartis), lincomycin (Pharmacia/Upjohn), acetylisovaleryltylosin (Eco) according to NCCLS M11-A5 (2003). The antibacterial substance to be tested a series of two-fold dilutions in WCABA. Pure cultures of *B. pilosicoli* were scraped from TSBA using sterile cotton swabs, and suspended in 2ml of sterile PBS. The turbidity was adjusted to the 1 Mc Farland standard. Twenty microlitres of the working suspension, which was prepared by a ten-fold dilution, was applied to the agar surface, which brought the final inoculum on the agar surface to approximately 105 CFU per spot. Each dish was inoculated with six isolates in spots distributed evenly over the surface in a rosette-like pattern. After a three-day incubation at 37°C, the result was read as the MIC, i.e. the lowest concentration of the drug tested that prevented the growth and haemolysis of the isolate on the inoculated spot. Each isolate was tested repeatedly twice. The values obtained for each of the drugs tested were used for the computation of MIC_{50}, MIC_{90} and the range of MICs.

Results and Discussion

The distribution of MICs for the *B. pilosicoli* isolates over the periods investigated is given in Table 1. MICs of tiamulin and valnemulin remained practically unchanged in the period of 2001-03 compared to the one of 1996-99. Increased MIC of pleuromutilins were recorded mostly on pig farms with swine dysentery history. Similar MICs of tiamulin for *B. pilosicoli* isolates were also reported from Finland and USA[4,5]. It follows from the results of the studies listed that *B. pilosicoli* is similar to *B. hyodysenteriae* in that it exhibits generally high MICs of AIVT and lincomycin[1,2]. Compared with AIVT and lincomycin, the prospects of the pleuromutilins in the therapy of PIS looked very promising.

Table 1. MICs for 33 Czech *B. pilosicoli* isolates over 1997-1999 and 2001-2003 periods

Antimicrobial agents	1997-1999 (n = 18)			2001-2003 (n = 15)			Total (n = 33)		
	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range
Tiamulin	0.125	2.0	0.03- 8.0	0.250	1.0	0.03-1.0	0.125	1.0	0.03- 8.0
Valnemulin	0.125	2.0	0.03-4.0	0.125	1.0	0.03-2.0	0.125	2.0	0.03-4.0
AIVT	100	200	3.125-200	50	200	3.125-200	50	200	3.125-200
Lincomycin	32	64	0.5-128	2.0	32	0.5-128	16	64	0.5-128

AIVT - acetylisovaleryltylosin

Acknowledgements

This study was supported by the Ministry of Education of the Czech Republic research project MSM 161700001.

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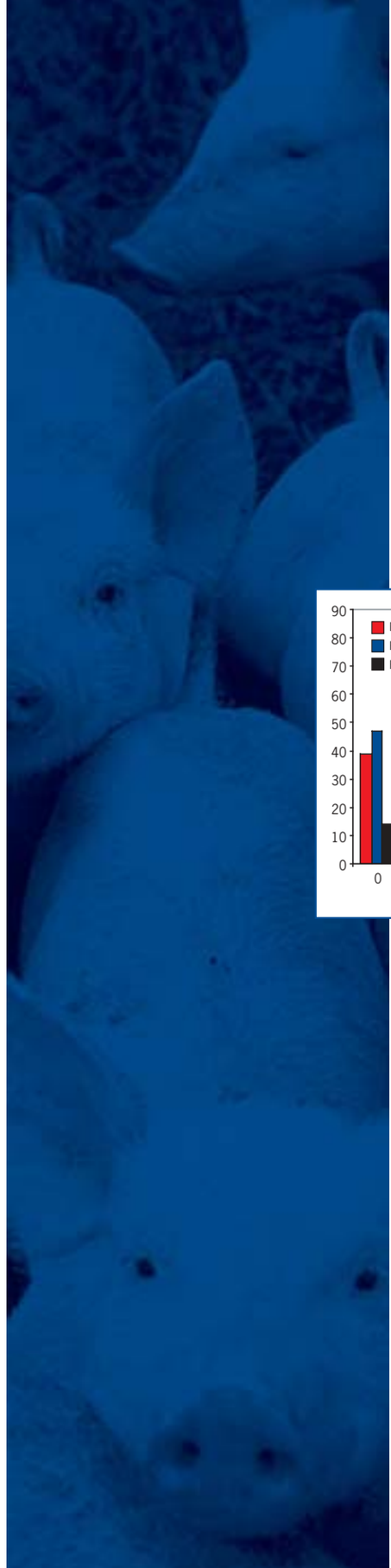


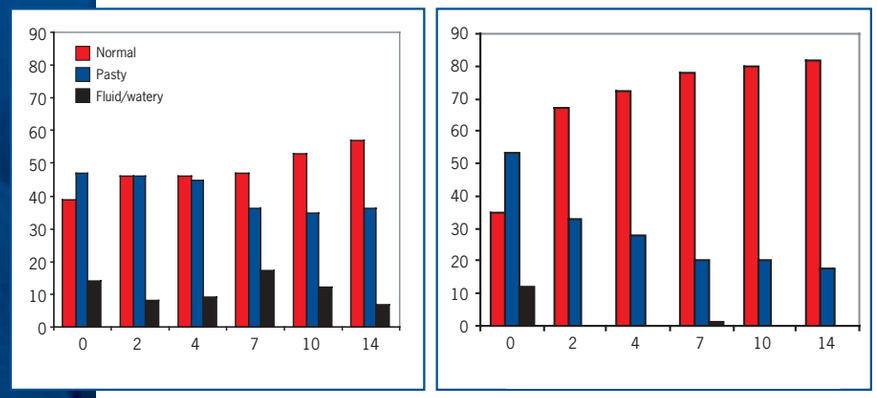
Efficacy of tiamulin (Tiamutin®) premix in the treatment of PPE in a field trial

A field trial in Germany showed that Tiamutin at treatment dosage (150ppm) was highly effective in the treatment of a naturally-occurring outbreak of Porcine Proliferative Enteropathy (PPE).

Table 1. Performance results

	Untreated control	**Tiamutin**	p-value
No. of pigs	74	**79**	
Daily weight gain (g)	530	**908 (+71%)**	0.0001

Figure 2. Faecal score results (pigs in %) in the untreated (left) and treated (right) group



Key facts

- *Tiamutin at an in-feed level of 150ppm for 14 consecutive days reduced significantly the severity and duration of diarrhoea in diseased pigs.*
- *The therapeutic response occurred rapidly.*
- *The significant improvement (+71%) in the weight gain of the medicated pigs corresponded to the positive effect of Tiamutin on the evaluated clinical parameters.*
- *The trial data confirm the high efficacy of Tiamutin Premix against Porcine Proliferative Enteropathy and indicate its positive effect on the performance of PPE-affected pigs.*

Efficacy of tiamulin (Tiamutin®) premix in the treatment of PPE in a field trial

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Introduction

Porcine proliferative enteropathy (PPE, ileitis) is a common enteric disease caused by *Lawsonia intracellularis*. The purpose of the study was to assess the efficacy of Tiamulin in the treatment of a naturally-occurring outbreak of PPE by monitoring different clinical parameters.

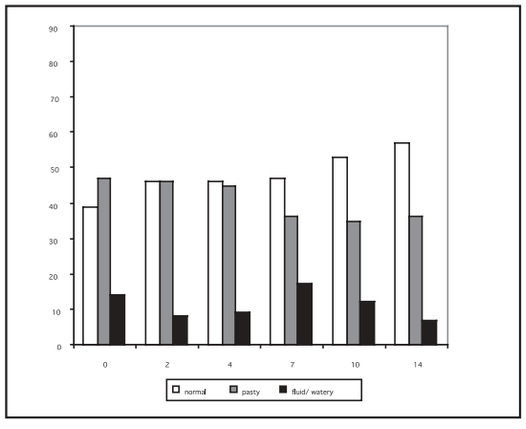
Materials and methods

The trial was a double blinded test. Pigs were naturally infected with *L. intracellularis* and were tested negative for the presence of Salmonella spp., *Brachyspira hyodysenteriae* and *B. pilosicoli*. The parasitological investigation was also negative. Two groups were stabled in a standard fattening building. Group A (n=79) was the untreated control group. Group B (n=76) was treated with tiamulin (Tiamutin®) at a dosage of 150 ppm for a period of 14 days. The pigs were monitored for 2 weeks and individually scored on day 0, 2, 4, 7, 10, 14 for the parameters clinical appearance, general condition, faecal score and presence of blood and/or mucus in the faeces. The medication period started, when at least 12% of the animals showed fluid or watery faeces. Faeces samples were taken from all animals with diarrhoea and examined for the presence of *L. intracellularis* (PCR). Blood samples were taken after the treatment period for serological examination. Weight gains and feed intake were compared between the treatment groups.

Results

At the beginning of the trial faeces scores in both treatment groups were classified as similar. Two days after the start of the therapy diarrhoea was successfully controlled by tiamulin. None of the pigs in group B showed diarrhoea in comparison to those in group A (p=0.0063), which continued to be sick (Fig. 1). The number of diseased pigs in group A stayed constant. The number of animals in this group with abnormal faeces consistency varied. In the minority of the cases pigs with abnormal faeces were observed on more than two days.

Figure 1. Percentage of pigs with different faecal scores in untreated group (top) and the medicated group (bottom)



Pigs medicated with tiamulin (Tiamutin®) showed improved daily weight gains during the trial period and higher bodyweights at the end of the trial. The mean weight gain in group B (908g/day) was significantly (p=0.0001) higher than in group A (530g/day) (Table 1).

Table 1: Body weights and weight increase from day 0-14

group	n	day 0	day 14	mean increase
A	74	35.1 kg	42.4 kg	7.4 kg
B	79	35.8 kg	48.5 kg	12.7 kg

No significant differences were found in the clinical appearance scores between both treatment groups during the trial. The general condition of pigs in group B improved during the trial period, in group A it worsened (p=0.0004). Single PPE infection of the herd was confirmed by PCR testing before onset of the trial and concurrent infections with other pathogens were excluded. During the trial serological assays (IFA) were used and the serology results showed the production of *L. intracellularis* antibodies in the serum of the tested pigs.

Discussion

In this trial the infection with *L. intracellularis* was confirmed by serological tests. Tiamulin has been reported to possess good in vitro activity against *L. intracellularis* [1,2]. The pronounced efficacy of tiamulin has been demonstrated in different artificial infection studies[3,4]. The therapeutic response to tiamulin (Tiamutin®) fed at a dosage of 150ppm was rapid. Significant improvements were observed in the clinical parameters faecal score and general condition. The severity and duration of diarrhoea were significantly reduced in pigs medicated with tiamulin. The improvement in the weight gain and feed consumption indicate the positive response to the tiamulin treatment. The trial also shows that financial losses during a PPE infection in growers are mainly caused by lower rates of weight gain and reduced food utilisation. They are not caused by an increased mortality. All these results lead to the conclusion that tiamulin feed at a dosage of 150ppm is effective in treating a naturally-occurring outbreak of PPE and can prevent economic losses associated with this disease.

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L.H. Nielsen

Attempt to eradicate Lawsonia intracellularis by medication in three sow herds

Tiamutin® Premix was used in three pig farms in Denmark to successfully eradicate Porcine Proliferative Enteropathy. The eradication strategy on the three farms was identical.

After depopulation followed by cleaning and disinfection, gilts from *Lawsonia*-positive farms were purchased and medicated during two successive time periods with Tiamutin (1st treatment: 14 days, dose: 8mg/kg bw/day followed by 2nd treatment: 14 days, dose 4mg/kg bw/day). A total treatment duration of 28 days was chosen in order to protect pigs from re-infection from the *Lawsonia*-contaminated farm environment.

After completion of the eradication programme the three farms were monitored in the following years for the presence of *Lawsonia intracellularis* (faeces/blood samples) and the pigs were clinically examined once per month. No antibiotic growth promoters were used on the farms.

Key facts

- ***Tiamutin successfully eliminated Lawsonia intracellularis from the three pig farms.***
- ***One (farm3), three (farm2) and four (farm1) years after completion of the eradication programmes, no clinical signs of ileitis infection occurred.***
- ***Faeces and blood samples taken on the farms during the monitoring period revealed freedom from Lawsonia intracellularis infection.***
- ***The trial data confirm that coupled with hygiene and management control measures, Porcine Proliferative Enteropathy can be eradicated by the application of Tiamutin® Premix.***

Attempt to eradicate *Lawsonia intracellularis* by medication in three sow herds.

L. Harm Nielsen
Ø-VET, Rude, Denmark.

Introduction and Objectives

Porcine proliferative enteropathy, caused by *Lawsonia intracellularis*, is a common disease in Denmark. Porcine proliferative enteropathy, is causing serious economic losses due to diarrhoea and reduced daily weight gain in weaner and grower pigs. Only a few studies are available regarding attempts to eradicate *Lawsonia intracellularis* from the farms[1, 2]. The objective of this study was to eradicate *Lawsonia intracellularis* from three farms established after total depopulation.

Materials and Methods

The eradication programme was carried out in three conventional farms. The total depopulation of the farm followed by cleaning and disinfection of the empty barns with glutar aldehyde. Rodent control was carried out and the barn was emptied for a period of 2-3 months. Gilts were purchased in all three farms from farms where *Lawsonia intracellularis* was demonstrated in the faeces by polymerase chain reaction (PCR) and the diagnosis was made by post mortem investigation. All new animals entering the farm were medicated with Tiamutin (tiamulin). The gilts entering the farm were over 50 kg (**Farm 1**: 225 gilts; **Farm 2**: 130 gilts and **Farm 3**: 280 gilts) and eradication strategies in all 3 farms were identical and implied medication with tiamulin through 2 periods separated by movement and washing of the animals: 14 days the gilts were treated with Tiamutin 2% premix, 12 kg/ton feed (corresponding to 8 mg/kg bodyweight/day) and then 14 days treatment with Tiamutin 2% premix, 6 kg/ton feed (corresponding to 4 mg/kg bodyweight/day). Between these two treatments pigs were moved to other pens and had their hooves cleaned and disinfected with a Virkon S solution of 2% to avoid transmission of *Lawsonia intracellularis* infection from the skin and hoofs of the pigs to the new cleaned and disinfected pens. After the eradication had been carried out all new purchased gilts were kept in quarantine stable until they were allowed into the farm. During the quarantine they went through the same medication procedure as described above. The eradication programme was carried out in **Farm 1** in August 1999, in **Farm 2** in May 2000 and in **Farm 3** in December 2002. After the eradication programme had been carried out, faeces samples were taken from weaners and growers in Farm 1 as shown in table 1. The number of samples was between 20 and 50 per date. In February 2004 30 blood samples were tested for specific antibodies for *Lawsonia intracellularis* by newly developed ELISA test at the Danish Institute for Food and Veterinary Research. For **Farm 2** and **Farm 3** faeces samples were taken once after eradication with 40 samples from weaners and growers. Faeces were tested for *Lawsonia intracellularis* by PCR. Subsequently, **Farms 1, 2** and **3** were clinically examined once per month after the eradication programme had been carried out. After the eradication programme was completed **Farms 1, 2** have 250, 130 sows respectively with production of 30 kg pigs. **Farm 3** has 280 sows with production of 30 kg pigs and finisher. No antibiotic growth promoters have been or are used in **Farms 1, 2** or **3**.

Results and Discussion

The eradication programme seems to have been successful. To date, in **Farms 1-3** four, three and one years after completing the eradication programme, respectively, there have been no clinical signs of *Lawsonia intracellularis* infection. As appears from Table 1 all faeces samples taken at different times from **Farm 1**, a total of 329, were negative for *Lawsonia intracellularis*. All 30 blood samples were found negative for *Lawsonia intracellularis* by indirect ELISA test. In **Farm 2** 40 faeces samples were taken on 14.10.2003 more than three years after eradication was carried out and all were negative for *Lawsonia intracellularis*. In **Farm 3** 40 faeces samples were taken on 10.10.2003 ten months after eradication was carried out and all were negative for *Lawsonia intracellularis*.

A total treatment duration of 28 days was chosen due to investigations showing that *Lawsonia intracellularis* can survive in the farm environment for up to 14 days. Therefore, with shorter treatment periods there was a risk that the pigs could be reinfected due to the survival of bacteria in the farm. The rationale for choosing tiamulin was previous experimental and field studies showing that tiamulin can eliminate *Lawsonia intracellularis* from the infected pigs[1, 3, 4].

Table 1. Monitoring of **Farm 1** for *Lawsonia intracellularis* in faeces from weaners and growers by PCR after eradication programme was carried out

Date	Number of faeces samples	Investigation for Lawsonia in faeces by PCR
03.10.2001	20	Not detected
21.01.2002	50	Not detected
06.02.2002	50	Not detected
04.11.2002	39	Not detected
21.01.2003	50	Not detected
27.02.2003	50	Not detected
18.08.2003	20	Not detected
23.01.2004	50	Not detected

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C. Glossop

Evaluation of the efficacy of Tiamutin® (tiamulin) in the treatment of naturally occurring Porcine Colonic Spirochaetosis

In a blinded and randomized field study in UK the efficacy of Tiamutin Premix (dose: 100ppm thf = 5mg/kg bw/day; application period 10 days) in pigs affected by colitis caused by *B. pilosicoli* alone was evaluated. Freedom from other enteric pathogens (*Brachyspira hyodysenteriae*, *Salmonella*, *Lawsonia*, *Yersinia*, *E. coli*) was confirmed by PCR-tests prior to the start of the trial. No growth promoters were used in the feed in either of the treatment groups.

	Untreated control	Tiamutin	p-value
No. of pigs	172	**168**	
Daily weight gain (g)	440	**515 (17% impr.)**	0.001
FCR	2.08	**1.88 (10% impr.)**	0.135

Key facts

- *Tiamutin Premix at a level of 5mg/kg bw/day improved significantly the clinical condition of pigs affected by colitis and prevented weight gain depression.*
- *A high sensitivity of the tested B.pilosicoli strains to tiamulin (MIC 0.025 mcg/ml) was found.*
- *The significant reduction ($p<0.001$) in the clinical scores and the significantly ($p<0.001$) lower number of pigs affected by colitis in the medicated group demonstrate the pronounced therapeutic effect of Tiamutin.*
- *The daily gain in the Tiamutin group was increased (17%) and the feed conversion ratio improved (10%).*
- *The trial data confirm the high efficacy of Tiamutin against colitis infections caused by Brachyspira pilosicoli.*

Evaluation of the efficacy of Tiamutin® (tiamulin) in the treatment of naturally occuring Porcine Colonic Spirochaetosis

C. Glossop
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Introduction and Objectives

In recent years, there has been an increase in the recognition of diarrhoea and poor performance in weaner and grower pigs associated with colitis (Porcine Colonic Spirochaetosis) (PCS) caused by *Brachyspira pilosicoli*. PCS typically affects pigs between 6-16 weeks of age, causing diarrhoea, decreased weight gain and feed conversion efficiency[1]. The objective of the study was to determine the clinical efficacy of tiamulin (Tiamutin®) in the feed, in pigs with colitis due to *Brachyspira pilosicoli* infection.

Materials and Methods

Clinical trials were carried out on a farm with approximately 280 sows. The pigs reared on the farm were sold at 30kg bodyweight. Clinical signs of colitis had been present in the grower pigs for some time, and the presence of *Brachyspira pilosicoli* had been confirmed. The trial farm was free from swine dysentery and ileitis. The diagnosis was confirmed by demonstration by culture of *Brachyspira pilosicoli* from diarrhoea faecal samples just before the start of the trial. At the time of the commencement of the study, 12 pens of pigs showing clinical signs of colitis were available for the study, 340 pigs in total, all of which were included in the trial. The pigs were housed on solid floors on straw within a grower house with natural ventilation. Pens of pigs were randomised immediately before the study commenced (Day 0), each pen being assigned to one of two groups based on the mean weight of pigs within each pen and the number of clinically affected pigs present, to ensure similar numbers of pigs with colitis were present in each group. The pens of pigs were assigned to one of two groups, one group to receive feed containing Tiamutin 2% Premix at a rate of 100ppm to achieve a dose of 5mg tiamulin/kg bodyweight, and one group to receive feed containing no medication (Control). Medication was continued for a total of 10 days. There were no growth promoters in the feed in either group.

All pigs were individually weighed and tagged on the day before the introduction of medicated feed (Day -1) and were weighed again on Day 8 and Day 11. All pigs received ad lib dry feed via hoppers. Feed consumption was recorded per pen. The study was blinded and all clinical observations were made by the Investigator who was unaware of the treatment allocation. Pigs were individually examined and scored on Day 0, Day 1, Day 3, Day 5, Day 7 and Day 10 for general appearance, general condition and faecal condition. Faecal samples were taken from 12 diarrhoeic pigs, one from each pen of pigs included in the study, immediately prior to the start of the trial for attempted isolation of *B. pilosicoli*, *B. hyodysenteriae*, *Salmonella spp.* and *Yersinia spp.* and detection of *Lawsonia intracellularis* by PCR. Weight gain and feed conversion rate were compared between treatment groups by Student's 't' test. The Mann-Whitney U test was used to compare the body condition, appearance and faecal condition and the overall response on Day 7 and Day 10.

Results and Discussion

The presence of *B. pilosicoli* was confirmed in 9 of the 12 samples that were taken on Day 0 of the study. Minimal inhibitory concentration (MIC) determination was carried out for 3 strains of *B. pilosicoli* taken at random from the 9 isolates and all 3 were highly susceptible to tiamulin with MIC values of 0.025µg/ml. *B. hyodysenteriae, L. intracellularis, Salmonella spp., Yersinia spp.* and *E. coli* were not detected from any of the samples. As appears from the table there was a significantly greater increase in average daily weight gain (ADG) (+17%) in the Tiamutin group compared to the Control group during the period of the study ($p = 0.001$). There was a numerical but not significant difference in Feed Conversion Ratio (FCR) between groups during the period of the study ($p = 0.135$).

	Control (n=172)	Tiamutin (n=168)	p-value
ADG (g/day)	440	515	0.001
FCR	2.08	1.88	0.135

There was no significant difference in the faecal condition of the pigs between groups on Day 0 ($p = 0.177$), but there was a significant difference on Day 3 ($p = 0.025$) and on Day 5 ($p = 0.002$) in favour of the Tiamutin group. There was a significant reduction in combined clinical score in favour of the Tiamutin group on both Day 7 and Day 10 ($p < 0.001$). In addition, significantly fewer new cases of colitis were observed during the period Day 3 to Day 10 in the Tiamutin group compared to the Control group ($p < 0.001$). Tiamutin was highly effective in reducing clinical signs and prevention of weight gain depression associated with PCS under field conditions.

References
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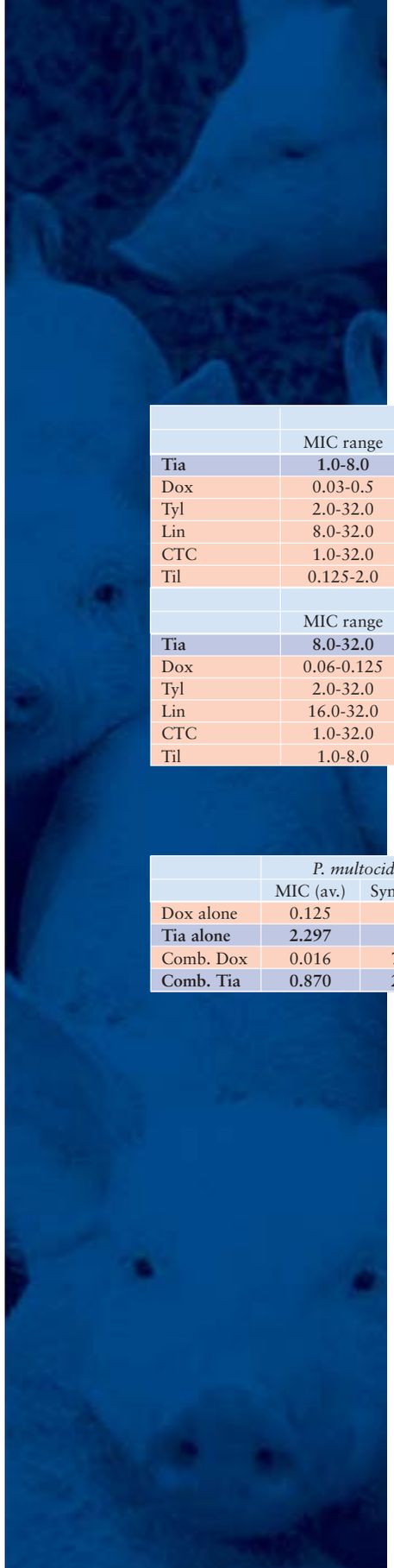

Sensitivity testing of respiratory swine pathogens to antimicrobials

The sensitivity of *Pasteurella multocida*, *Streptococcus suis*, *Bordetella bronchiseptica* and *Actinobacillus pleuropneumoniae* field strains was tested against different antibiotics.

Table 1. Range of MICs, MIC_{50} and MIC_{90} of the tested antibiotics for *P. multocida*, *S. suis*, *B. bronchiseptica* and *A. pleuropneumoniae* (µg/ml)

	P. multocida			*S. suis*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	**1.0-8.0**	**2.0**	**4.0**	**0.015-0.5**	**0.125**	**0.25**
Dox	0.03-0.5	0.125	0.25	0.125-8.0	0.125	0.125
Tyl	2.0-32.0	16.0	32.0	0.06-0.5	0.125	0.25
Lin	8.0-32.0	16.0	16.0	0.03-2.0	0.5	0.5
CTC	1.0-32.0	8.0	16.0	8.0-32.0	8.0	16.0
Til	0.125-2.0	1.0	2.0	0.03-2.0	1.0	2.0
	B. bronchiseptica			*A. pleuropneumoniae*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	**8.0-32.0**	**16.0**	**32.0**	**2.0-4.0**	**2.0**	**4.0**
Dox	0.06-0.125	0.06	0.125	0.25-8.0	0.25	1.0
Tyl	2.0-32.0	32.0	32.0	4.0-32.0	16.0	32.0
Lin	16.0-32.0	32.0	32.0	1.0-16.0	8.0	16.0
CTC	1.0-32.0	4.0	32.0	1.0-32.0	8.0	32.0
Til	1.0-8.0	4.0	8.0	0.5-2.0	2.0	2.0

Table 2. Average MIC values of tiamulin and doxycycline alone and in combination

	P. multocida		*S. suis*		*B. bronchiseptica*		*A. pleuropneumoniae*	
	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor
Dox alone	0.125	-	0.189	-	0.088	-	0.435	-
Tia alone	**2.297**	**-**	**0.094**	**-**	**16.0**	**-**	**2.297**	**-**
Comb. Dox	0.016	7.8 x	0.025	7.6 x	0.017	5.2 x	0.088	4.9 x
Comb. Tia	**0.870**	**2.6 x**	**0.044**	**2.1**	**5.656**	**2.8 x**	**1.071**	**2.1 x**

Key facts

- *Tiamulin is demonstrated in vitro to be more active against S. suis than a range of competitor products – lincomycin, tilmicosin, CTC, doxycycline.*
- *The trial data prove the synergistic activity of tiamulin in combination with doxycycline against respiratory pathogens.*
- *The synergistic activity against P. multocida, B. bronchiseptica, A. pleuropneumoniae and S. suis strains resulted in remarkable enhancement of the antibacterial activity and in 2.1-7.8 times lower MIC values.*
- *The new in vitro results are consistent with previously generated data demonstrating the synergism between tiamulin and tetracyclines.*
- *The combination of Tiamutin® and doxycycline successfully combats the wide range of bacterial pathogens involved in the respiratory disease complex. The combined application for the treatment of complex respiratory infections is clinically desirable.*

Sensitivity testing of respiratory pathogens of swine to antimicrobials

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Introduction

Current respiratory disease syndromes are often a multi-faceted complex with many infectious agents involved. Pathogens like *M. hyopneumoniae, A. pleuropneumoniae, P. multocida, B. bronchiseptica* and *S. suis* play a role in the induction of these disease syndromes. Proper protection during multi-pathogen attacks is based on the application of antimicrobials.

Objectives

The aim of this study was to test the sensitivity of the above-mentioned bacterial pathogens of swine origin to valnemulin, tiamulin, tylosin, lincomycin, tilmicosin, chlortetracycline, doxycycline, and to combinations of valnemulin+doxycycline and tiamulin+doxycycline.

Material and methods

Ten strains of the bacterial species were freshly isolated from the lung and other organs of swine. The isolates were identified biochemically following the description by Barrow and Feltham (1993). The sensitivity test was performed in phenol red dextrose broth. From each antibiotic, valnemulin-hydrochloride, tiamulin hydrogen fumarate (Novartis AH), doxycycline-hydrochloride, chlortetracycline-hydrochloride, lincomycin-hydrochloride, tylosin tartrate (Fluka), tilmicosin (Elanco), a solution containing 124µg was prepared, filtered through 450nm, Millipore. Sterile solutions were stored at –20°C until used.

The tests were performed in Polystyrene microplates. Duplicate doubling dilutions of antibiotics were made from 32 to 0.03µg/ml. Inoculum was 103 CFU/ml. The plates were sealed with cellotype and incubated at 37°C over a period of 24-48 hours. The test was read when the control wells showed bacterial growth. The reading was performed on 3 consecutive days. The lowest concentrations of the antibiotics completely preventing growths of bacteria were considered to be the MIC (µg/ml). Sensitivity of the strains was also tested against combinations of valnemulin+doxycycline and tiamulin+doxycycline.

Results and conclusions

Range of MICs, MIC_{50} and MIC_{90} are presented in Table 1 and 2. Concentrations of the drugs alone and in combinations are presented in Table 3.

Using combinations of valnemulin/tiamulin+doxycycline, concentrations of the drugs required to produce inhibitory effects could be reduced significantly, in comparison with the MIC values exhibited by the antibiotics used singly (synergy factor 2.1-9.5). The results suggest that the combined use may be clinically useful in the treatment of complex respiratory infections in swine.

References
Barrow G.I. and Feltham, R.K.A. (1993) *Cowan and Steel's Manual for the identification of medical bacteria.* Cambridge Univ. Press, Cambridge.

Table 1. Range of MICs, MIC_{50} and MIC_{90} of tested antibiotics for *P. multocida* and *S. suis*

	P. multocida			*S. suis*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	1.0-8.0	2.0	4.0	0.015-0.5	0.125	0.25
Dox	0.03-0.5	0.125	0.25	0.125-8.0	0.125	0.125
Val	1.0-4.0	2.0	4.0	0.015-0.125	0.03	0.03
Tyl	2.0-32.0	16.0	32.0	0.06-0.5	0.125	0.25
Lin	8.0-32.0	16.0	16.0	0.03-2.0	0.5	0.5
CTC	1.0-32.0	8.0	16.0	8.0-32.0	8.0	16.0
Til	0.125-2.0	1.0	2.0	0.03-2.0	1.0	2.0

Table 2. Range of MICs, MIC_{50} and MIC_{90} of tested antibiotics for *B. bronchiseptica* and *A. pleuropneumoniae*

	B. bronchiseptica			*A. pleuropneumoniae*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	8.0-32.0	16.0	32.0	2.0-4.0	2.0	4.0
Dox	0.06-0.125	0.06	0.125	0.25-8.0	0.25	1.0
Val	8.0-16.0	8.0	16.0	2.0-4.0	2.0	4.0
Tyl	2.0-32.0	32.0	32.0	4.0-32.0	16.0	32.0
Lin	16.0-32.0	32.0	32.0	1.0-16.0	8.0	16.0
CTC	1.0-32.0	4.0	32.0	1.0-32.0	8.0	32.0
Til	1.0-8.0	4.0	8.0	0.5-2.0	2.0	2.0

Table 3. MIC values of antibiotics alone/in combinations

	P. multocida		*S. suis*		*B. bronchiseptica*		*A. pleuropneumoniae*	
	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor
Dox alone	0.125	-	0.189	-	0.088	-	0.435	-
Val alone	1.741	-	0.023	-	11.31	-	2.143	-
Comb. Dox	0.016	7.8	0.02	9.5	0.010	8.8	0.143	3.0
Comb. Val	0.435	4.0	0.011	2.1	4.287	2.6	0.870	2.5
Dox alone	0.125	-	0.189	-	0.088	-	0.435	-
Tia alone	2.297	-	0.094	-	16.0	-	2.297	-
Comb. Dox	0.016	7.8	0.025	7.6	0.017	5.2	0.088	4.9
Comb. Tia	0.870	2.6	0.044	2.1	5.656	2.8	1.071	2.1

D. Taylor

Treatment of APP infection by water medication with tiamulin (Tiamutin®)

A challenge study was carried out with *Actinobacillus pleuropneumoniae* and the effect of Tiamutin water medication on clinical and performance parameters was determined.

Table 1. Clinical findings

	Unmedicated control	**Tiamutin**
No. of pigs	6	**6**
Weight gain (kg)	-3.5	**+0.5**
Daily water intake (ml)	390	**1002**
Feed intake (total in kg)	8.0	**17.0**
Days APP	13/21 (38%)	**5/36 (14%)**
Days fever	12/24 (50%)	**13/42 (31%)**
Mortality	3/6 (50%)	**0**

Table 2. Postmortem findings

	Unmedicated control	**Tiamutin**
No. of pigs died	3	**0**
Extent of APP lesions	38%	**1.9%**
Tiamulin conc. normal lung	n.d.	**1.3 – 22.3mcg/ml**
Tiamulin conc. pneumonic lung	n.d.	**5.0 – 15.5mcg/ml**

n.d. – not detected

Key facts

- *Tiamutin at levels of 120ppm (0.012% in the water) effectively reduced clinical signs and APP-specific lung lesions.*
- *Positive clinical effects of Tiamutin included improved growth rate and substantially increased water and feed intake in the APP-affected animals.*
- *Tiamulin achieved inhibitory concentrations in the lung.*
- *The tiamulin concentrations in the diseased lungs (5-15.5mcg/ml) exceeded the MIC value of the Actinobacillus strain used (3mcg/ml) 1.7-5.1 times.*
- *Tiamutin in drinking water can be used for the successful treatment of pleuropneumonia.*

Treatment of *Actinobacillus pleuropneumoniae (APP)* infection by water medication with tiamulin

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Introduction

A qualitative study was carried out in 1990 to evaluate water medication with tiamulin in the treatment of experimental pleuropneumonia in the pig. It is presented here because the findings illustrate and explain some aspects of field experience with tiamulin in the treatment of the disease.

Materials and methods

12 weaned pigs free from pleuropneumonia, were weighed, divided into two groups of six and housed in separate pens in the same airspace. Feed and water were freely available. Challenge infection was carried out with *Actinobacillus pleuropneumoniae* isolate 6664, serotype 3, which was pathogenic for pigs. Ten ml of a 3 hour shaken culture of the organism grown in Trypticase Soy Broth with added NAD was given intranasally on one occasion only. Clinical examination for respiratory and enteric disease was carried out preinfection and daily thereafter and the results recorded. Rectal temperatures were recorded. Pen 1 received unmedicated water and Pen 2 received water medicated with tiamulin 0.012%. Feed and water consumption were recorded daily from 44 hours post infection. Animals *in extremis* were euthanased and all remaining animals were killed 8 DPI.

Post mortem examination included mapping the lesions present in the lung and weighing the respiratory tract. Bacteriological and histological examinations were carried out on the lungs from all pigs. MIC determinations were carried out on strain 6664, 9 reisolated strains and 4 isolates of *Pasteurella multocida*. Levels of tiamulin present in plasma, normal and pneumonic lung were determined using biological assay with *Micrococcus luteus* in agar preincubated for 18 hours. Zone sizes produced by standards were used to construct standard curves and calibrated. The levels in tissue samples were then determined.

Results

Infection was successful and animals either died of pleuropneumonia or developed clinical signs typical of the disease.

Three pigs in Pen 1, the untreated control group died or were killed. The remaining animals had clinical signs of pleuropneumonia but began to recover from 3 DPI onwards. The treated group (Pen 2) were found to have some exercise intolerance, but there were no deaths and they all maintained body condition. The clinical findings are summarised in Table 1.

Table 1. Clinical findings

	Pen 1	Pen 2
Treatment	None	Tiamulin 0.012%
Pig numbers	6	6
Weight gain	-3.5 kg	+0.5kg
Daily water used	390 ml	1002 ml
Total feed intake	8.0kg	17.0kg
Days App	13/21 (38%)	5/36 (14%)
Days fever	12/24 (50%)	13/42 (31%)
Mortality	3/6 (50%)	0

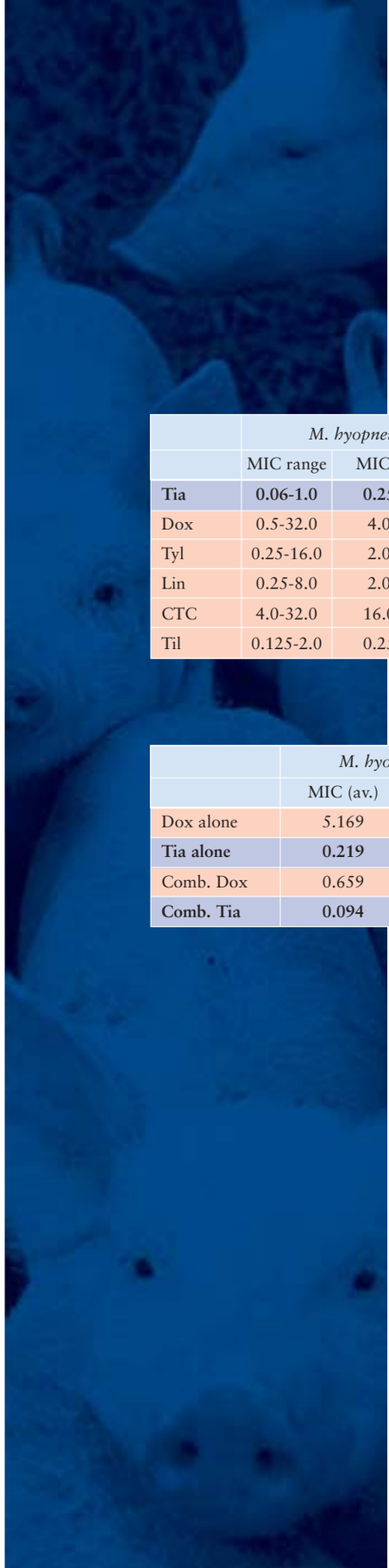
Lesions of pleuropneumonia were present in pigs from both pens, but those in the untreated controls were markedly more severe than those from the treated group (Table 2).

P. multocida was isolated from the lungs of all untreated controls but from only one of the treated pigs. MICs of the inocular strain and all reisolates of *A. pleuropneumoniae* were 3.0µg/ml. Those of *P. multocida* were in excess of 10µg/ml.

Table 2. Postmortem findings

	Pen 1	Pen 2
Treatment	None	Tiamulin 0.012%
Number died	3	0
Lung weight	445 g	235g
Lesion extent	38%	1.9%
Plasma level Tiamulin	Not Done	0.0-0.20µg/ml.
Tiamulin level normal lung	Not Done	1.3-22.3µg/ml.
Tiamulin level pneumonic lung	Not Done	5.0-15.5µg/ml.

It was concluded that pigs affected by pleuropneumonia might not drink or eat normal amounts and that water medication with tiamulin could treat disease successfully by preventing mortality and reducing production loss and lung lesions. Levels in diseased lung exceeded the MIC of the organism used.



L. Stipkovits and others

Sensitivity testing of Mycoplasma pathogens to tiamulin (Tiamutin®) and other antimicrobials

The sensitivity of Hungarian strains of *M. hyopneumoniae*, *M. hyorhinis* and *M. hyosynoviae* to tiamulin, the combination of tiamulin and doxycycline and to other antimicrobials was tested.

Table 1. MIC range, MIC_{50} and MIC_{90} of the tested antibiotics for *M. hyopneumoniae*/*M. hyosynoviae*/*M. hyorhinis* (µg/ml)

	M. hyopneumoniae			*M. hyosynoviae*			*M. hyorhinis*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	**0.06-1.0**	**0.25**	**1.0**	**0.03-0.25**	**0.125**	**0.25**	**0.06-2.0**	**0.125**	**1.0**
Dox	0.5-32.0	4.0	16.0	0.25-4.0	1.0	2.0	0.06-8.0	0.5	8.0
Tyl	0.25-16.0	2.0	16.0	2.0-32.0	4.0	16.0	4.0-32.0	8.0	32.0
Lin	0.25-8.0	2.0	8.0	0.5-8.0	2.0	4.0	1.0-8.0	2.0	8.0
CTC	4.0-32.0	16.0	32.0	8.0-32.0	8.0	32.0	4.0-32.0	16.0	32.0
Til	0.125-2.0	0.25	2.0	2.0-32.0	4.0	16.0	0.5-8.0	2.0	8.0

Table 2. Average MICs of tiamulin and doxycycline alone and in combination

	M. hyopneumoniae		*M. hyosynoviae*		*M. hyorhinis*	
	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor
Dox alone	5.169	-	1.101	-	0.933	-
Tia alone	**0.219**	-	**0.120**	-	**0.219**	-
Comb. Dox	0.659	7.8 x	0.116	9.5 x	0.094	9.9 x
Comb. Tia	**0.094**	**2.3 x**	**0.044**	**2.7 x**	**0.116**	**1.9 x**

Key facts

- *The Mycoplasma strains tested showed high sensitivity to tiamulin.*
- *The MIC_{90} values of doxycycline, tylosin, lincomycin, chlortetracycline and tilmicosin were between 2-128 times higher than those of tiamulin.*
- *The MIC data confirm the exceptional potency of tiamulin against Mycoplasma hyorhinis and Mycoplasma hyosynoviae.*
- *Tiamulin combined with doxycycline possesses a synergistic activity.*
- *The synergistic activity of tiamulin and doxycycline against the M. hyopneumoniae, M. hyorhinis and M. hyosynoviae strains resulted in 1.9-9.9 times lower MIC values.*
- *The new in vitro results are consistent with previously generated Mycoplasma-related data and the clinical response to Tiamutin® observed in the field and in many in vivo studies.*

Sensitivity testing of *Mycoplasma* pathogens to antimicrobials

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Introduction

Mycoplasmal pneumonia and arthritis are widespread all over the world and cause significant economical losses. The use of antibiotics is one part of integrated mycoplasma disease control programmes and based on periodical evaluation of the sensitivity of the mycoplasma strains being present on the pig units.

Objectives

The aim of the studies was to test the sensitivity of mycoplasma strains of swine origin belonging to *Mycoplasma hyopneumoniae, M. hyorhinis* and *M. hyosynoviae* species to valnemulin, tiamulin, tylosin, lincomycin, tilmicosin, chlortetracycline, doxycycline, and to combinations of valnemulin+doxycycline and tiamulin+doxycycline.

Material and methods

Ten strains of each bacterial species were isolated from the lung of swine using Friis medium (Friis, 1975) or medium B (Erno and Stipkovits, 1973). They were cloned, identified biochemically (Stipkovits et al., 1973) and serologically. The sensitivity test was performed in the same media. From each antibiotic (valnemulin-hydrochloride, tiamulin hydrogen fumarate (Novartis AH), doxycycline-hydrochloride, chlortetracycline-hydrochloride, lincomycin-hydrochloride, tylosin tartrate (Fluka), tilmicosin (Elanco)), a solution containing 124µg was prepared, filtered through 450nm, Millipore. Sterile solutions were stored at –20°C until used.

The sensitivity tests were performed in Polystyrene microplates (Tanner and Wu, 1992). Duplicate doubling dilutions of antibiotics were made from 32 to 0.03µg/ml. The inoculum was 105 CFU/ml. The plates were sealed with cellotype and incubated at 37°C over a period of 2-6 days. The plates were observed every day. The test was read first when the phenol red indicator in the control wells turn orange (to pH = 7.0) in case of *M. hyopneumoniae* and *M. hyorhinis* strains or pink (to pH = 8.0) in case of *M. hyosynoviae* strains. Evaluation was repeated 1 and 2 days after the first reading. The lowest concentrations of the antibiotics completely preventing colour change of the media at the third reading were considered to be the MIC(µg/ml). Sensitivity of the strains was also tested against combinations of valnemulin+doxycycline and tiamulin+doxycycline.

Results and discussion

Range of MICs, MIC_{50} and MIC_{90} are presented in Table 1. Concentration of drugs alone and in combinations are shown in Table 2.

The tested mycoplasma strains showed the highest susceptibility to valnemulin and tiamulin. The trial results prove the synergistic activity of valnemulin and tiamulin against mycoplasma pathogens, when used in combination with doxycycline (synergy factor 1.2-12.3).

References
Erno H. and Stipkovits, L. (1973) Il. Acta. Vet. Scand. 14: 450-463.
Stipkovits L. et al. (1973) Acta Vet. Hung. 23: 307-313.
Friis N.F. (1975) Nord. Vet. Med. 25: 337-339.
Tanner A.C. and Wu C.C. (1992) Av. Dis. 36: 714-717.

Table 1. MIC range, MIC_{50} and MIC_{90} of the tested antibiotics for *M. hyopneumoniae/hyosynoviae/hyorhinis*

	M. hyopneumoniae			*M. hyosynoviae*			*M. hyorhinis*		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Tia	0.06-1.0	0.25	1.0	0.03-0.25	0.125	0.25	0.06-2.0	0.125	1.0
Dox	0.5-32.0	4.0	16.0	0.25-4.0	1.0	2.0	0.06-8.0	0.5	8.0
Val	0.06-1.0	0.06	0.5	0.015-0.125	0.06	0.125	0.06-0.5	0.06	0.25
Tyl	0.25-16.0	2.0	16.0	2.0-32.0	4.0	16.0	4.0-32.0	8.0	32.0
Lin	0.25-8.0	2.0	8.0	0.5-8.0	2.0	4.0	1.0-8.0	2.0	8.0
CTC	4.0-32.0	16,0	32.0	8.0-32.0	8.0	32.0	4.0-32.0	16.0	32.0
Til	0.125-2.0	0.25	2.0	2.0-32.0	4.0	16.0	0.5-8.0	2.0	8.0

Table 2. MIC values of antibiotics alone/in combinations

	M. hyopneumoniae		*M. hyosynoviae*		*M. hyorhinis*	
	MIC (av.)	Syn. fact.	MIC (av.)	Syn. fact.	MIC (av.)	Syn. fact.
Dox alone	5.169	-	1.101	-	0.933	-
Val alone	0.120	-	0.069	-	0.091	-
Comb. Dox	0.466	11.1	0.116	9.5	0.076	12.3
Comb. Val	0.101	1.2	0.020	3.5	0.041	2.2
Dox alone	5.169	-	1.101	-	0.933	-
Tia alone	0.219	-	0.120	-	0.219	-
Comb. Dox	0.659	7.8	0.116	9.5	0.094	9.9
Comb. Tia	0.094	2.3	0.044	2.7	0.116	1.9

Plasma and tissue kinetic study of tiamulin (Tiamutin®) in pigs

A pharmacokinetic study was conducted to determine the concentration of tiamulin in key target tissues of the pig, e.g. lung, colon mucosa and colon contents, following single intramuscular injection (15mg/kg tiamulin hydrogen fumarate = 12.2mg/kg tiamulin base).

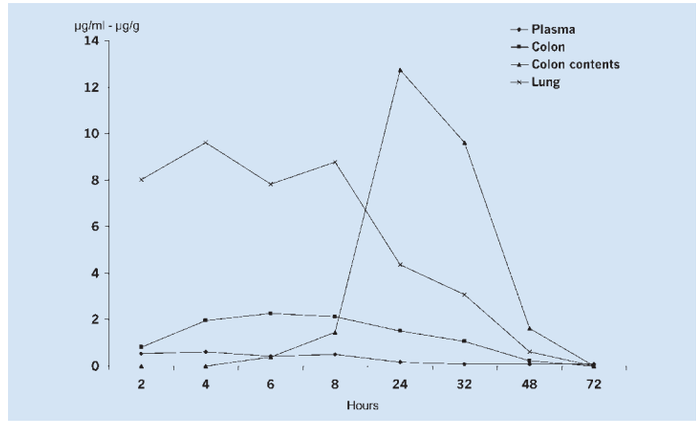

Table 1. Pharmacokinetics of tiamulin in different tissues

	Plasma	Colon mucosa	Colon content	Lung
AUC (mcg.h/ml)	12.82	64.51	314.23	231.52
AUMC (mcg.h²/ml)	252.02	1252.78	9013.0	3868.1
MRT (h)	19.66	19.42	28.68	16.71
Cmax (mcg/ml)	0.61	2.27	12.75	9.6
Tmax (h)	4.0	6.0	24.0	4.0

Key facts

- ***Constant tiamulin lung tissue levels (8mcg/ml) were achieved between 2 and 8 hours p.m. The levels were still greater than 3mcg/ml at 32 hours p.m.***
- ***Very high tiamulin concentrations in the colon contents were detected at 24 hours (12.7mcg/ml) and 32 hours (9.6mcg/ml) p.m.***
- ***The mean concentration of tiamulin in the colon mucosa rose to a maximum (2.27mcg/ml) at six hours p.m.***
- ***The tiamulin concentrations in the lung tissue, colon mucosa and colon contents are in excess of the MIC values of many respiratory (Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae, Mycoplasma hyosynoviae) and enteric (Brachyspira hyodysenteriae, Lawsonia intracellularis) pathogens.***

Plasma and tissue kinetic study of tiamulin (Tiamutin®) in pigs

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Introduction and Objectives

The objective of the study was to determine the plasma and target tissue distribution of tiamulin or its metabolites in pigs using a microbiological assay.

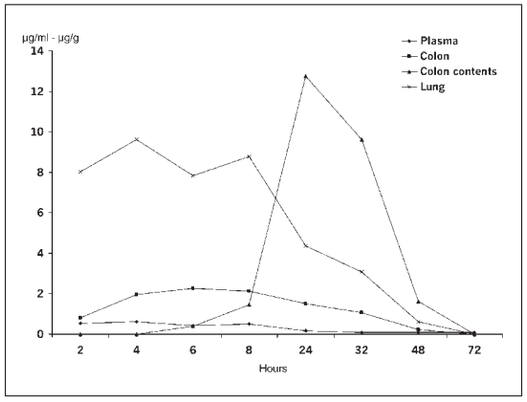
Materials and Methods

Twenty-five male Landrace X pigs 20 to 25 kg were used. The animals were acclimatised for one week before the start of the study and were fed a non-medicated weaner/grower cereal diet. The pigs were numbered randomly and administered 15 mg/kg bodyweight Tiamutin 200 injection (Tiamulin expressed as the hydrogen fumarate salt by intramuscular injection) in the left dorsalateral aspect of the neck. Three pigs were killed at each of the following time points, 2, 4, 6, 8, 24, 32, 48 and 72 h after administration of the tiamulin. One pig was not administered tiamulin and was killed with the 2 h pigs to supply blank plasma and tissue and to act as an untreated control. Blood was taken and lung tissue, colon wall, colon content and, where possible, synovial fluid and bronchial fluid were collected. All samples were stored frozen at -20°C until analysis. Plasma and tissue samples were analysed by antimicrobial assay using *Sarcinia lutea* (NCIMB No.5883) as the test organism.

The precision of the agar gel diffusion assay of tiamulin was good as demonstrated by the low values for the within-day and between-day coefficients of variation of zone diameter (<3.0% and <5.0% respectively). The linearity of the zone diameters evaluated from replicate standards was good (r =0.998). Calculations of sample concentrations were made by comparison with a standard curve prepared from fortified blank tissue. The method expresses total antimicrobial activity which may be attributed to tiamulin or to its metabolites. The mean maximum plasma concentration (Cmax) and time of Cmax(tmax) were determined from observed values. The area under the plasma concentration time curve (AUC) and area under the moment curve (AUMC) were determined using the trapezoidal rule from observed data using a Microsoft excel spreadsheet package and the mean residence time (MRT) was calculated as AUMC/AUC.

Results and Discussion

Figure 1.



The mean tiamulin serum and tissue levels are shown in Fig.1. The concentration of tiamulin in lung tissue of pigs were very much larger than in plasma. The ratio of mean lung:plasma concentrations were between 15:1 and 19:1 from 2 to 8 hours after administration and were even larger at 24 hours and 32 hours after administration. Mean lung concentrations were quite constant at approx. 8 µg/ml between 2 and 8 hours after administration and were still greater than 3 µg/ml at 32 hours after administration. The mean concentration of tiamulin in colon wall rose to a maximum 2.27 µg/ml at 6 hours after administration and the drug was still present at >1 µg/ml 32 hours after administration. Tiamulin could not be detected in the colon contents at 2 or 4 hours after administration and mean concentrations were < 1.5 µg/ml at 6 and 8 hours. Very high concentrations were, however, detected at 24 hours (12.7 µg/ml) and 32 hours (9.6 µg/ml). The Cmax and AUC values were very much higher for colon wall, colon contents and lung than for plasma (Table 1).

Table 1. Pharmacokinetics of tiamulin in tissues calculated from mean concentrations from each time of kill.

	Plasma	Colon Wall	Colon Contents	Lung
AUC (µg.h/ml)	12.82	64.51	314.23	231.52
AUMC (µg.h^2/ml)	252.02	1252.78	9013.00	3868.10
MRT (h)	19.66	19.42	28.68	16.71
Cmax (µg/ml)	0.61	2.27	12.75	9.60
tmax (h)	4.00	6.00	24.00	4.00

Concentrations occurred later in colon content than other tissues indicating that excretion of the drug occurs by the enteric route.

Measurement of bronchial fluid and synovial fluid concentrations was possible only from four pigs. Two pigs had tiamulin concentration in bronchial fluid at 6 and 8 hours of 3.90 and 2.53 µg/ml respectively and another two pigs had tiamulin concentration in synovial fluid, at 4 and 8 hours of 0.19 and 0.38 µg/ml, respectively. This study shows that the concentrations achieved with the dose of 15 mg/kg bodyweight of tiamulin following i.m. injection, in the lung, colon and colon contents exceed the MIC values for key respiratory and enteric pathogens such as *Brachyspira hyodysenteriae, Lawsonia intracellularis, M. hyopneumoniae, M. hyosynoviae* and many strains of *Actinobacillus pleuropneumoniae*.

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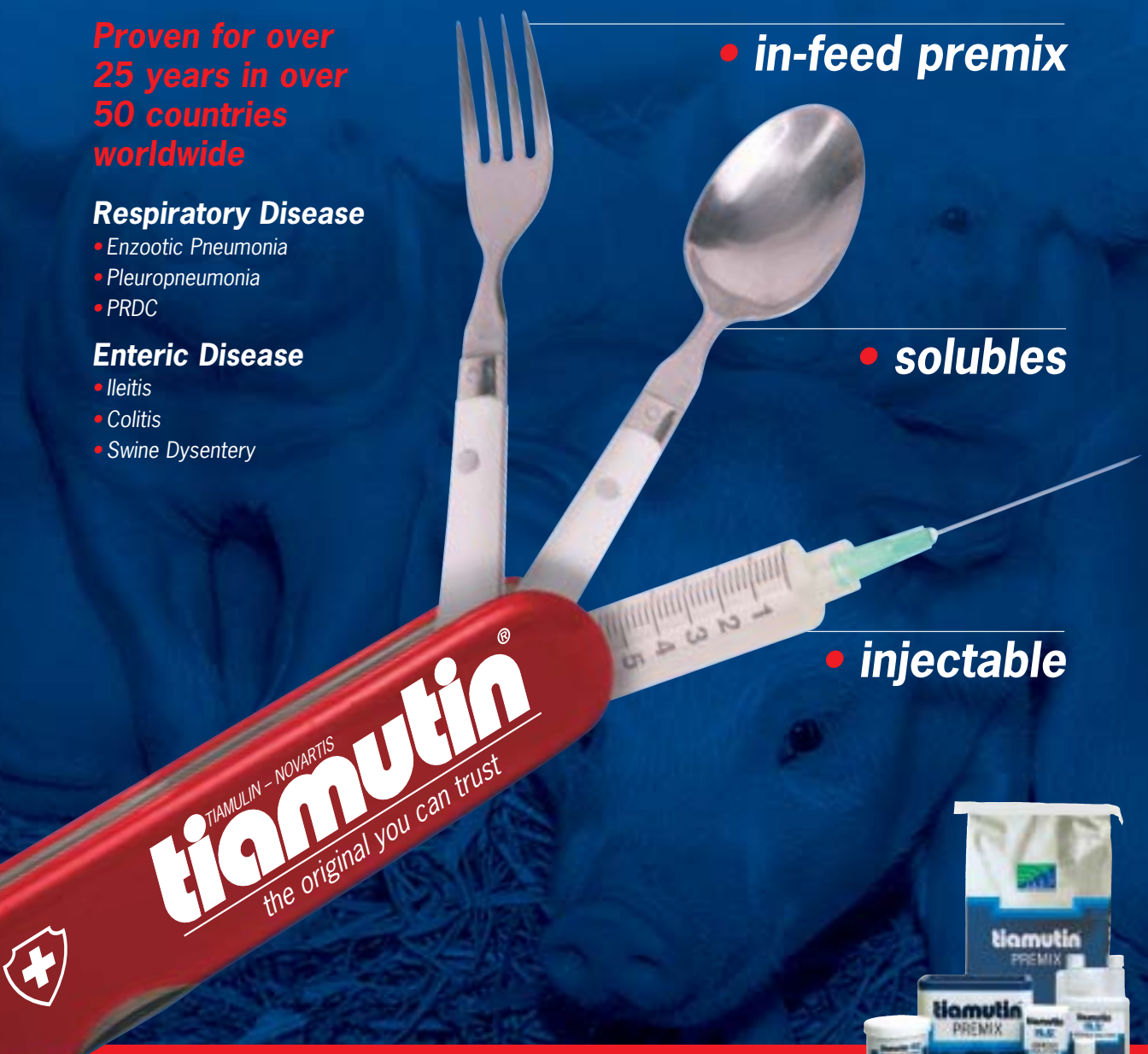

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