



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

VALNEMULIN  
**ECONOR**<sup>®</sup>  
THE NEXT GENERATION PREMIX



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This Book of Proceedings – a collection of papers relating to ECONOR presented during the 18th IPVS Congress, Hamburg – is further proof of Novartis Animal Health's continued commitment to the global pig industry and it is intended to give pig veterinarians valuable assistance in the creation of their pig health and production strategies.

**Dr. Clive Girdler**  
Global Category  
Manager  
– Pig Products

**Dr. Ulrich Klein**  
International Technical  
Services Manager  
– Pig Products

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## TRENDS IN RESISTANCE PATTERNS OF BRACHYSPIRA PILOSICOLI TO VALNEMULIN (ECONOR®) IN EUROPE AND THE USA

The antimicrobial susceptibility of *B. pilosicoli* strains isolated between 1990-1997 and 1997-2003 to valnemulin was compared and the trend of resistance development evaluated.

Table 1: Results of MIC determinations ( $\mu\text{g/ml}$ ) carried out on isolates obtained 1990-1997

Study Country	Date isolation	n	MIC range	Geo-metric mean	MIC <sub>50</sub>	MIC <sub>90</sub>
Moller 1996* DK	1994-1995	5	0.0156	0.016	0.016	0.016
Kinyon 2002 USA	1990-1995	16	0.03 – 0.06	0.06	0.06	0.06
Kessler 2001 DE	1991-1997	6	0.063 – 0.5	0.25	0.25	0.5
Dalziel 1997 UK	1995-1996	12	<0.015 – 0.125	0.02	0.018	0.125

\* weakly B-haemolytic spirochaetes presumed to be *S. pilosicoli*; DE = Germany; DK = Denmark

Table 2: Results of MIC determinations ( $\mu\text{g/ml}$ ) carried out on isolates obtained 1997-2003

Study Country	Date isolated	n	MIC range	Geo-metric mean	MIC <sub>50</sub>	MIC <sub>90</sub>
Kessler 2001 DE	1998-2000	7	0.125 – 0.25	0.15	0.125	0.25
Merialdi 2003 Italy	2001-2003	10	<0.067 – 2.0	0.793	0.25	1.0
Mars 2003 UK	2002-2003	10	0.0075 – 0.25	0.037	0.04	0.25
Kinyon 2002 USA	1997-1999	9	0.03 – 2.0	0.27	0.5	1.0

### KEY FACTS

- Low valnemulin MIC values were found during both evaluated time periods.
- The MIC data confirm the exceptional potency of valnemulin against *Brachyspira pilosicoli*.
- The trial results show little trend towards increasing MICs in *B. pilosicoli* and no evidence of significant resistance development.
- The results support the use of ECONOR against Porcine Colonic Spirochaetosis (PCS) at correct dose levels.

# Trends in resistance patterns of *Brachyspira pilosicoli* to valnemulin (Econor®) in Europe and the USA

P.H. Ripley<sup>1</sup>, U. Klein<sup>2</sup>, A. Mars<sup>3</sup>, G. Merialdi<sup>4</sup>, P. Bonilauri<sup>4</sup> and M. Kessler<sup>5</sup>

<sup>1</sup>Novartis Animal Health, Litlington, U.K. <sup>2</sup>Novartis Animal Health, Basel, Switzerland. <sup>3</sup>Veterinary Laboratories Agency, Itchen Abbas, U.K., <sup>4</sup>Instituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy, <sup>5</sup>Institute of Microbiology, School of Veterinary Medicine, Hannover, Germany.

## Introduction

The development of resistance in animal pathogens is of obvious concern to both veterinary and human medicine. Disease becomes less responsive to treatment, and the potential for cross-resistance is increased.

Econor (valnemulin) was developed in the mid 1990s, initially for the control of enzootic pneumonia caused by *Mycoplasma hyopneumoniae*, and the prevention and treatment of swine dysentery caused by *Brachyspira hyodysenteriae*. It was launched in Europe in 1999.

Very recently it has received approval for the control of ileitis (*Lawsonia intracellularis*) and colitis (*B. pilosicoli*). Since Minimum Inhibitory Concentrations (MICs) of valnemulin for *B. pilosicoli* isolates were measured both in the mid 1990s and more recently, the opportunity presented itself for a comparison of MIC values obtained then and now.

## Materials and Methods

MIC determinations were made by introducing *B. pilosicoli* isolates into replicate agar plates containing valnemulin at a range of doubling dilutions. Normally *B. pilosicoli* and *B. hyodysenteriae* standards were concurrently set up as controls. The plates were incubated and the MIC recorded as the lowest concentration of valnemulin which prevented visible growth (Kessler: haemolysis) on the plates.

## Results

Results of earlier determinations are set out in Table 1, and later results in Table 2. MICs are expressed as µg/ml.

Table 1. Results of MIC determinations carried out on isolates obtained 1990-1997.

Study Country	Date isolation	n	MIC range	Geo-metric mean	MIC <sub>50</sub>	MIC <sub>90</sub>
Moller 1996* DK <sup>1</sup>	1994-1995	5	0.0156	0.016	0.016	0.016
Kinyon 2002 USA <sup>3</sup>	1990-1995	16	0.03 – 0.06	0.06	0.06	0.06
Kessler 2001 DE <sup>2</sup>	1991-1997	6	0.063 – 0.5	0.25	0.25	0.5
Dalziel 1997 UK	1995-1996	12	<0.015 – 0.125	0.02	0.018	0.125

\*weakly B-haemolytic spirochaetes presumed to be *S. pilosicoli*; DE = Germany; DK = Denmark

Table 2. Results of MIC determinations carried out on isolates obtained 1997-2003.

Study Country	Date isolated	n	MIC range	Geo-metric mean	MIC <sub>50</sub>	MIC <sub>90</sub>
Kessler 2001 DE	1998-2000	7	0.125 – 0.25	0.15	0.125	0.25
Merialdi 2003 Italy	2001-2003	10	<0.067 – 2.0	0.793	0.25	1.0
Mars 2003 UK	2002-2003	10	0.0075 – 0.25	0.037	0.04	0.25
Kinyon 2002 USA	1997-1999	9	0.03 – 2.0	0.27	0.5	1.0

No obvious trends emerge, and differences between countries are most likely to be due to inter-laboratory variation. In some cases MICs were determined using both agar and broth dilution methods. Results on agar, used here, tended to be a dilution higher than those obtained in broth.

The results of Dalziel 1997 and Mars 2003; and also of Kinyon and Kessler are of particular interest since they allow comparisons of values obtained in the same laboratory.

## Discussion and Conclusions

These results show little trend towards increasing MIC or resistance development in *B. pilosicoli*. Resistance development in spirochaetes is of course possible. Karlsson et al.<sup>4</sup> have recently shown this to be possible, although the rate of development was very slow. *B. hyodysenteriae* resistance against different therapeutics has been reported in the field from the Czech Republic by Cizek et al.<sup>5</sup> but it is known that animals are often medicated at lower doses than those recommended.

The development of resistance can be minimised by thorough treatment of clinical disease at correct treatment dose levels, and by strategic medication at prevention levels to prevent or reduce environmental contamination.

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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 ( $\mu\text{g/ml}$ ) 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 <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range
<b>ECONOR®</b> (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
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
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 valnemulin and tiamulin 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 valnemulin and tiamulin isolated between 2001-2003 remained practically unchanged in comparison to those four years ago.
- The data confirm the low sensitivity of *B. pilosicoli* to both AIVT and lincomycin.
- Valnemulin and tiamulin 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*

A. Cizek, D. Sperling, D. Lobova, J. Smola

Institute of Microbiology and Immunology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic.

## 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<sup>7</sup>. 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), acetylisovaleryltylosin (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<sup>3</sup> and PCR-RFLP<sup>6</sup>. The type strains of *B. hyodysenteriae* B78<sup>T</sup> (ATCC 27164<sup>T</sup>), *B. innocens* B256<sup>T</sup> (ATCC 29796<sup>T</sup>), *B. pilosicoli* P43/6/78 (ATCC 51139<sup>T</sup>) and *B. murdochii* 56-150<sup>T</sup> (ATCC 51284<sup>T</sup>) 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<sub>50</sub>, MIC<sub>90</sub> 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<sup>4,5</sup>. It follows from the results of the studies listed that *B. pilosicoli* is similar to *B. hyodysenteriae* in that it exhibits a generally high MICs of AIVT and lincomycin<sup>1,2</sup>. Compared with AIVT and lincomycin, the prospects of the pleuromutilins in the therapy of PIS looked very promising.

## Acknowledgements

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

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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 <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	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

C. Alexopoulos and others

## THE EFFICACY OF ECONOR® (VALNEMULIN HYDROCHLORIDE) FOR THE PREVENTION OF ILEITIS IN WEANERS AGAINST NEGATIVE AND POSITIVE (TYLOSIN) CONTROLS

The efficacy of ECONOR for the prevention of ileitis postweaning was compared to a positive and negative control in a farrow-to-finish farm and the performance of the pigs during and beyond the medication period was evaluated.

Table 1: Control of the clinical signs of ileitis during/beyond the medication period

Period/Parameter	Control	Tylan	ECONOR
<b>Nursery stage:</b>			
Mean diarrhoea score	1.771 <sup>a</sup>	0.729 <sup>b</sup>	0.243 <sup>c</sup>
Pig morbidity (pos. samples/total in %)	27/96 (28.1%) <sup>a</sup>	10/96 (10.4%) <sup>b</sup>	6/96 (6.3%) <sup>c</sup>
<b>Grower stage:</b>			
Mean diarrhoea score	8.694 <sup>a</sup>	3.842 <sup>b</sup>	1.387 <sup>c</sup>
Pig morbidity (pos. samples/total in %)	40/94 (42.6%) <sup>a</sup>	24/94 (25.5%) <sup>b</sup>	11/94 (11.7%) <sup>c</sup>

<sup>a,b,c</sup> Means/percentages in a row with different superscripts differ (p<0.05)

Table 2: Performance beyond the medication period and calculated cost benefit during the evaluation period

Period/Parameter	Control	Tylan	ECONOR
<b>Nursery stage:</b>			
Start weight/ End weight (kg)	7.42/23.27	7.46/23.97	7.50/24.21
ADG (g)	453 <sup>b</sup>	472 <sup>a</sup>	478 <sup>a</sup>
FCE	1.34 <sup>a</sup>	1.26 <sup>b</sup>	1.24 <sup>b</sup>
<b>Grower stage:</b>			
Start weight/ End weight (kg)	23.27/50.15	23.97/51.93	24.21/52.99
ADG (g)	549 <sup>a</sup>	571 <sup>b</sup>	587 <sup>a</sup>
FCE	2.87 <sup>a</sup>	2.76 <sup>b</sup>	2.67 <sup>c</sup>
Cost benefit (Euro/ pig) weaning until end grower stage	- - -	1.90	3.14

<sup>a,b,c</sup> Means in a row with different superscripts differ (p<0.05)

### KEY FACTS

- Single application of ECONOR for 21 days at 50ppm postweaning effectively controlled ileitis during and beyond the medication period (evaluation period 12 weeks).
- Efficient disease control post-medication resulted in statistically improved daily gain and feed conversion in ECONOR-medicated pigs compared to those in the Tylan group (100ppm).
- The trial data prove the excellent clinical efficacy and long-term effect of ECONOR against ileitis.
- The improvement of the zootechnical performance throughout the trial period resulted in a cost benefit which was calculated to 3.14 Euro/pig and 1.90 Euro/pig, respectively in ECONOR- and Tylan-treated pigs, compared with the negative controls.

# The efficacy of valnemulin hydrochloride for the prevention of ileitis in weaners against negative and positive (tylosin) controls

C. Alexopoulos<sup>1</sup>, E.D. Tzika<sup>1</sup>, I.E. Georgoulakis<sup>2</sup>, S. Lekkas<sup>1</sup>, E. Bourtzi-Hatzopoulou<sup>1</sup>, S.C. Kyriakis<sup>1</sup>

<sup>1</sup>School of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Macedonia, Greece; <sup>2</sup>School of Agriculture, Animal Production and Hydatid Environment, University of Thessaly, 384 46 Volos, Greece.

## Introduction and Objectives

Ileitis control has been based mainly on the use of certain antimicrobials, which have been found to be effective either in challenge exposure studies<sup>1</sup> or field trials<sup>2,3</sup>. The aim of this study was to document the efficacy of valnemulin hydrochloride given for a three-week period postweaning via feed (50 ppm), compared with both negative controls and positive controls (100 ppm tylosin).

## Materials and Methods

The trial was carried out in a farrow-to-finish pig farm with history of ileitis. Random faecal samples were taken from 60 pigs with diarrhoea, prior to the beginning of the trial, among different pens and ages for microbiological examinations (*Salmonella spp.* and *Brachyspira hyodysenteriae*) and PCR (*Lawsonia intracellularis, LI*). Moreover, 30 intestine samples were taken from pigs that were slaughtered according to the farm practices at different ages for PCR (*Brachyspira pilosicoli*). All samples were negative for all bacteria examined except for *LI*. The prevalence of *LI* ranged from 6.67% (at weaning) to 66.67% (at slaughter age). A total of 288 piglets on the day of weaning (25±1.5 days of age) were individually weighed and allocated to three experimental groups (96/group). Within each group, 4 replicates (pens) of 24 piglets were used. The piglets of the first group were treated as negative controls, while in the feed of the remaining two groups 500 mg Tylan 2% (Elanco Animal Health) and 50 mg Econor premix 10% (Novartis Animal Health Inc.), were added, respectively. Medicated feed was offered to the piglets from weaning and for a period of 21 days. Piglets were monitored daily for disease signs from weaning and for a total of

13 weeks. A diarrhoea score (DS) was calculated separately for the nursery stage (NS) and the growing stage (GS). Pigs were weighed individually at the end of each period (NS: 61±1.5 days of age and GS: 110±1.5 days of age). On the same days rectal swabs for PCR test (*LI*) were obtained from the same 4 piglets per pen. Feed consumption per pen was recorded on a weekly basis. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Data was analysed with ANOVA including pen, sex and experimental group using the GLM procedure of SAS (release 8.2 for WINDOWS – 2001).

## Results and Discussion

The results are presented in Tables 1 to 5. No significant differences were noticed among the groups relative to piglet mortality, or to ADFI. The results clearly demonstrated a beneficial effect of both valnemulin hydrochloride (VH) and tylosin (TY) for the control of ileitis in weaners, as shown by the lower morbidity due to ileitis and the lower DS in treated piglets. As a result, FCR was also improved in both treatment groups during the NS. Furthermore, it suggests that VH, had a more long term effectiveness than TY. Even during the GS (several weeks after the treatments) lower diarrhoea scores and FCR were noticed in VH treated animals, probably due to a more pronounced lowering of *LI* shedding in the environment, as also indicated by PCR results. Finally, the cost benefit, based on the improvement of zootechnical parameters throughout the whole trial period (weaning to the end of GS) was calculated to 3.14 €/pig and 1.90 €/pig, respectively in VH and TY treated animals, compared with the negative controls.

Table 1. PRC in rectal swabs (positive samples/total examined, %)

Time	Experimental groups		
	Controls	Tylosin	Valnemulin
Weaning	1/16 (6.3%) <sup>a</sup>	1/16 (6.3%) <sup>a</sup>	1/16 (6.3%) <sup>a</sup>
End of NS	4/16 (25.0%) <sup>a</sup>	1/16 (6.3%) <sup>ab</sup>	0/16 (0.0%) <sup>b</sup>
End of GS	7/16 (43.8%) <sup>a</sup>	4/16 (25.0%) <sup>a</sup>	2/16 (12.5%) <sup>a</sup>

<sup>ab</sup> Percentages in a row with different superscripts differ (P<0.05).

Table 5. Mean (± SD) FCR of trial pigs

Period	Experimental groups		
	Controls	Tylosin	Valnemulin
NS	1.34 <sup>a</sup> ±0.03	1.26 <sup>b</sup> ±0.03	1.24 <sup>b</sup> ±0.03
GS	2.87 <sup>a</sup> ±0.05	2.76 <sup>b</sup> ±0.04	2.67 <sup>c</sup> ±0.03
Total	2.30 <sup>a</sup> ±0.02	2.21 <sup>b</sup> ±0.03	2.14 <sup>c</sup> ±0.02

<sup>abc</sup> Means in a row with different superscripts differ (P<0.05).

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Table 2. Pig morbidity (positive samples/total examined, %)

Period	Experimental groups		
	Controls	Tylosin	Valnemulin
NS	27/96 (28.1%) <sup>a</sup>	10/96 (10.4%) <sup>b</sup>	6/96 (6.3%) <sup>b</sup>
GS	40/94 (42.6%) <sup>a</sup>	24/94 (25.5%) <sup>b</sup>	11/94 (11.7%) <sup>c</sup>

<sup>abc</sup> Means in a row with different superscripts differ (P<0.05).

Table 3. Mean (± SD) diarrhoea score of trial pigs

Period	Experimental groups		
	Controls	Tylosin	Valnemulin
NS	1.771 <sup>a</sup> ±0.340	0.729 <sup>b</sup> ±0.179	0.243 <sup>c</sup> ±0.187
GS	8.694 <sup>a</sup> ±0.469	3.842 <sup>b</sup> ±0.602	1.387 <sup>c</sup> ±0.603

<sup>abc</sup> Means in a row with different superscripts differ (P<0.05).

Table 4. Mean (± SD) ADG of trial pigs

Period	Experimental groups		
	Controls	Tylosin	Valnemulin
NS	0.453 <sup>b</sup> ±0.057	0.472 <sup>a</sup> ±0.043	0.478 <sup>a</sup> ±0.031
GS	0.549 <sup>c</sup> ±0.065	0.571 <sup>b</sup> ±0.053	0.587 <sup>a</sup> ±0.043
Total	0.509 <sup>b</sup> ±0.054	0.529 <sup>a</sup> ±0.041	0.542 <sup>a</sup> ±0.029

<sup>abc</sup> Means in a row with different superscripts differ (P<0.05).

U. Klein and others

## PENETRATION OF VALNEMULIN (ECONOR®) INTO ENTEROCYTES

A trial was conducted in order to study the ability of valnemulin to penetrate enterocytes. Rat enterocyte cell cultures were used for this study which offer identical metabolic conditions in comparison to porcine enterocytes.

Table 1: Valnemulin (Val) concentration in the culture media and in the enterocytes after incubation with various concentrations for 4 and 24 hours

Sample flasks	Val conc	Incubation period	Val media conc. (µg/ml)	Val cell-suspension conc. (µg/g)
1	1µg	4 hours	0.355	4.21
2	1µg	4 hours	0.299	2.44
3	1µg	4 hours	0.297	4.43
4	1µg	24 hours	0.268	6.27
5	1µg	24 hours	0.254	7.32
6	1µg	24 hours	0.277	6.15
7	10µg	4 hours	2.73	27.6
8	10µg	4 hours	2.77	36.9
9	10µg	4 hours	2.76	38.2
10	10µg	24 hours	2.35	67.1
11	10µg	24 hours	2.23	75.5
12	10µg	24 hours	2.27	79.2
13	100µg	4 hours	29.3	198
14	100µg	4 hours	25.2	172
15	100µg	4 hours	28.6	155
16	100µg	24 hours	33.0	157
17	100µg	24 hours	31.8	196
18	100µg	24 hours	29.0	246

### KEY FACTS

- The results show that valnemulin is able to penetrate intestinal epithelial cells and to accumulate in cultured enterocytes.
- The data confirm previous studies which have shown the penetration and accumulation of valnemulin in *Lawsonia intracellularis* cells and the high inhibition of those pathogens (MIC <2µg/ml) based on the administration of ECONOR.

# Penetration of valnemulin (Econor®) into enterocytes

U. Klein<sup>1</sup>, P. Ripley<sup>2</sup>, S. Marshall<sup>3</sup>, C. Kikuta<sup>4</sup>

<sup>1</sup>Novartis Animal Health, Basel, Switzerland <sup>2</sup>Novartis Animal Health, Litlington, U.K., <sup>3</sup>BioBest Laboratories Ltd, Penicuik, U.K.,

<sup>4</sup>pharm-analyt Labor GmbH; Baden, Austria.

## Introduction

The usefulness of antimicrobials in the treatment of enteric infections caused by facultative/obligate intracellular bacteria may depend on the ability of the antimicrobial to enter, accumulate and persist in the enterocytes and to cause bacterial death. *Lawsonia intracellularis* (*L.i.*), the causative agent of porcine proliferative enteropathy (PPE, ileitis), colonizes the intestinal tract, enters the enterocytes and is located free in their cytoplasm. The purpose of the study was to study the ability of valnemulin to penetrate cultured enterocytes. This would serve as the basis for the efficacy of valnemulin against intracellular organisms such as *Lawsonia*.

## Materials and methods

Rat enterocytes were used in this study. Pig enterocytes are difficult to culture and there are currently no pig enterocyte cell lines readily available. Rat intestinal epithelial cells (cell line IEC-18; ECACC 88011801) were cultured using BioBest standard procedures. Stock valnemulin solution was added to the cell culture media to achieve final concentrations of 1, 10 and 100 µg/ml (6 flasks for each concentration). Three flasks from each concentration were harvested after 4 hours, the remaining 3 flasks after 24 hours incubation. After the incubation period (at 37°C, 5% CO<sub>2</sub>) the media from each flask was removed and stored at -20°C. The cells were washed (sterile PBS) and detached from the flask

(trypsin/versene). The cell suspension was then centrifuged and the supernatant was removed. The cell pellet and supernatant were stored at -20°C. After harvest of all the flasks an aliquot of the cells, the pooled PBS wash, the trypsin/versene solution and the culture media from each flask were sent to pharm-analyt for analysis of the valnemulin concentration using HPLC.

## Results

The concentration of valnemulin found in the culture medium confirmed the correct addition of the valnemulin solution to each flask. In blank cultures of enterocytes no valnemulin was found. Higher and dose-related concentrations were found in the cells in comparison to the culture media indicating the uptake of valnemulin. The valnemulin uptake in flasks 1-12 depended on the incubation time. An approximately 100% increase in the uptake of valnemulin between 4 and 24 hours was observed. Incubation with concentrations of 1 and 10µg/ml was reflected by a ten-fold increase in the uptake between flasks 1-6 and flasks 7-12. Such a difference was not observed between flasks 7-12 and 13-18. These results at the higher incubation dosages of 100µg/ml suggest a saturation of the uptake of valnemulin. Increased incubation times did not lead to any higher valnemulin concentrations in the enterocytes in flasks 13-18.

Table 1. Valnemulin (Val) concentration in the culture media and in the enterocytes after incubation with various valnemulin concentrations for 4 and 24 hours

Sample flasks	Val conc	Incubation period	Val media conc. (ug/ml)	Val cell-suspension conc. (µg/g)
1	1µg	4 hours	0.355	4.21
2	1µg	4 hours	0.299	2.44
3	1µg	4 hours	0.297	4.43
4	1µg	24 hours	0.268	6.27
5	1µg	24 hours	0.254	7.32
6	1µg	24 hours	0.277	6.15
7	10µg	4 hours	2.73	27.6
8	10µg	4 hours	2.77	36.9
9	10µg	4 hours	2.76	38.2
10	10µg	24 hours	2.35	67.1
11	10µg	24 hours	2.23	75.5
12	10µg	24 hours	2.27	79.2
13	100µg	4 hours	29.3	198
14	100µg	4 hours	25.2	172
15	100µg	4 hours	28.6	155
16	100µg	24 hours	33.0	157
17	100µg	24 hours	31.8	196
18	100µg	24 hours	29.0	246

cell concentration in the region of 199µg/g. The ability of valnemulin to penetrate and accumulate in *L.i.* cells, resulting in their inhibition, was shown in Minimum Inhibitory Concentration (MIC) studies (McOrist et al.<sup>2</sup> in which *L.i.* was co-cultured in enterocyte cell lines in the presence of valnemulin. The low MIC values (<2µg/ml) shown in these studies and the high in vivo efficacy of valnemulin found in field studies indicate that *Lawsonia* strains are highly susceptible to valnemulin.

The uptake of valnemulin by enterocytes is shown in this study and provides a rationale for the use of valnemulin in the effective control of *Lawsonia* infections.

## References

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2. McOrist S. et al. (1998) Proc. 16th IPVS Congress, Birmingham. 114.
3. McOrist S. et al. (1997) Vet. Microbiol. 54: 385-392.

## Discussion

Rat enterocyte cultures offer identical metabolic conditions and results generated in these cells are likely to apply to the situation in porcine enterocytes, Lawson et al.<sup>1</sup>, McOrist et al.<sup>3</sup>. The results show that valnemulin is able to penetrate intestinal epithelial cells and to accumulate in cultured enterocytes. The uptake is dose-dependent up to a maximum

M.A. Jones and others

## EFFICACY OF VALNEMULIN (ECONOR®) IN THE TREATMENT OF PORCINE PROLIFERATIVE ENTEROPATHY UNDER FIELD CONDITIONS

Two field trials were carried out to evaluate the efficacy of ECONOR given at treatment dosage for 14 days on farms with permanent ileitis problems.

### Trial 1

	Untreated control	ECONOR	p
Mean weight gain (kg)	7.37	8.77 (19% impr.)	<0.0001
Food Conversion Ratio	2.28	1.94 (15% impr.)	NS
Pigs with diarrhoea during trial	35 (46%)	8 (11%)	<0.0001
No. of PCR-positive samples during trial	12 (16%)	2 (3%)	<0.01

### Trial 2

	Untreated control	ECONOR	p
Mean weight gain (kg)	11.83	13.91 (17.6% impr.)	<0.005
Food Conversion Ratio	2.73	2.43 (11% impr.)	0.11
Cumulative faecal score during trial	460	396	<0.005

### KEY FACTS

- ECONOR at levels between 3.15-4.46 mg/kg bodyweight effectively treated pigs with ileitis under typical field conditions.
- ECONOR improved the growth rate and food conversion ratio substantially.
- The trial data confirm the high efficacy of ECONOR against *Lawsonia intracellularis*-based field infections.

# Efficacy of valnemulin (Econor®) in the treatment of porcine proliferative enteropathy (PPE) (Ileitis) under field conditions

M.A. Jones<sup>1</sup>, C.S. Gale<sup>2</sup>, P A Roger<sup>3</sup>, D.G.S. Burch<sup>4</sup>, P.H. Ripley<sup>5</sup>

<sup>1</sup>Leeds Veterinary Laboratories, Leeds, U.K., <sup>2</sup>Wood Veterinary Group, Gloucester, U.K., <sup>3</sup>Swale Veterinary surgery, Richmond, U.K.,

<sup>4</sup>Octagon Services, Old Windsor, U.K., <sup>5</sup>Novartis Animal Health, Litlington, U.K.

## Introduction

Valnemulin (Econor®), a pleuromutilin antibiotic, is highly effective in the prevention and treatment of swine dysentery (*Brachyspira hyodysenteriae*) (Holck et al.<sup>1</sup>), porcine colonic spirochaetosis (*B. pilosicoli*) (Morgan et al.<sup>2</sup>) and the control of enzootic pneumonia (*Mycoplasma hyopneumoniae*) (Ripley<sup>3</sup>). *Lawsonia intracellularis*, the causative agent of ileitis, is sensitive to valnemulin in vitro, and valnemulin's efficacy has been demonstrated in challenge studies (McOrist et al.<sup>4</sup>). Trials were conducted on farms where ileitis was an on-going problem but on which swine dysentery had not been recorded, in order to assess Econor's efficacy under field conditions.

## Materials and Methods

Trials were conducted on 2 farms, using 150 and 159 pigs respectively in 6 and 8 pens respectively on each farm. Both herds had a long standing clinical history of ileitis. At the start of the trial, pigs were weighed, scored for faecal state, demeanour and general condition and ranked according to faecal state and bodyweight. Pairs were formed and allocated to pens randomly assigned to Econor or placebo medication so that the number of pigs with diarrhoea, and the mean weight of pigs, was similar in treatment or placebo groups. The Econor for feed inclusion was supplied in coded sachets so that the Investigator, Study Monitor and farm staff were blinded with respect to the

identity of the treatments. The dosage achieved was 4.17 and 4.46mg/kg body weight on the first trial site, and 3.15mg/kg on the second.

Medication continued for 14 days, and faecal state and clinical condition were scored every 2-4 days. Faecal samples were taken from pigs with diarrhoea during the study and from a pre-identified sub-group at the start and end of the trial. These were examined for the presence of *L. intracellularis* by PCR testing. Pigs were weighed at the beginning and end of the trial, food consumed per pen recorded, and Food Conversion Ratios calculated.

## Results

Weights, diarrhoea and clinical scores were similar in both Econor and placebo groups at the start of the trial.

The number of pigs with diarrhoea and diarrhoea scores were reduced by Econor medication, particularly in the first trial. In this trial there was a high percentage of PCR-positive animals at the start of the trial and the number of positive samples was significantly reduced in the Econor group during the trial. The number of PCR-positive samples was lower at the start of the 2nd trial and declined in both groups. Demeanour and general condition scores were lower better in Econor-medicated groups in both trials. These reductions were accompanied by highly significant increases in mean weight gain, together with associated improvement in food conversion efficiency.

Table 1. Results from 1st Ileitis trial

	Econor	placebo	p
n	75	75	
Mean Wt. Gain (kg)	8.77	7.37	<0.0001
Food Conversion Ratio	1.94	2.28	NS
No. with diarrhoea* on Day 0	8 (11%)	8 (10%)	NS
No. with diarrhoea* during the trial	8 (11%)	35 (46%)	<0.0001
PCR positive samples Day 0	9/17 (53%)	7/27 (26%)	NS
Cumulative no. of PCR +ve samples during trial	2 (3%)	12 (16%)	<0.01

Table 2. Results from 2nd Ileitis trial

	Econor	placebo	p
n	79	80	
Mean Wt. Gain (kg)	13.91	11.83	<0.005
Food Conversion Ratio	2.43	2.73	0.11
No. with diarrhoea* on Day 0	20 (25%)	19 (24%)	NS
No. with diarrhoea* during the trial	24 (30%)	31 (39%)	NS
Cumulative faecal score during trial	396	460	<0.005

\* faecal score 2 or more (0 = normal, 3 = watery)

## Discussion and Conclusions

In the first trial, the prevalence of diarrhoea was lower at the start of the trial, rising in the placebo group but not in the Econor group. There was also a higher rate of positive PCR test results. In the 2nd trial, the level of diarrhoea was higher at the start of the study and the reduction due to medication less striking. On the other hand the number of PCR-positive faeces was lower. Pigs were older and heavier in the 2nd trial, disease more advanced and immunity probably developing. There may also have been a higher level of secondary infection in this trial and the dose of Econor was lower. Econor medication was effective in reducing the clinical signs of ileitis and reducing weight loss associated with ileitis under typical field conditions.

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J.I. Velásquez and S.Velásquez

## WEIGHT GAIN OF EIGHTY GROW-FINISH PIGS PROPHYLACTICALLY TREATED WITH TWO LEVELS OF TYLOSIN AND VALNEMULIN

In a farm with pneumo-enteric infections (Enzootic Pneumonia, Porcine Proliferative Enteropathy) located in ANTIOQUIA a randomized field study was carried out to evaluate the efficacy of ECONOR® (37.5ppm/75ppm) in comparison to Tylan® (55ppm/110ppm).

	ECONOR (37.5 ppm)	Tylan (55ppm)	ECONOR (75ppm)	Tylan (110ppm)
No. of pigs	20	20	20	20
Total weight gain (kg)	79.9 <sup>c</sup>	78.27 <sup>b</sup>	81.51 <sup>a</sup>	80.56 <sup>ac</sup>
FCE	2.41	2.53	2.37	2.42

<sup>a,b,c</sup> Means with the same letter are not different (p<0.000016)

### KEY FACTS

- The results indicate the high efficacy of ECONOR in the control of concurrent infections caused by *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* which are frequently encountered in the field.
- ECONOR at a dose of 75ppm had superior efficacy and improved the performance of the pigs compared to Tylan at both tested dose levels.
- ECONOR at 37.5ppm significantly improved the weight gain compared to Tylan at 55ppm.

# Weight gain of 80 grow-finish pigs prophylactically treated with two levels of tylosin and valnemulin

J.I. Velásquez<sup>1</sup>, S. Velásquez<sup>2</sup>

<sup>1</sup>School of Veterinary Medicine, University of Antioquia, Medellín, Colombia. <sup>2</sup>Novartis Animal Health, Bogota, Colombia.

## Introduction

World wide Enzootic Pneumonia caused by *Mycoplasma hyopneumonia* and Porcine Proliferative Enteritis (PPE) caused by *Lawsonia intracellularis* are two very prevalent diseases during the grow-finish phase of pig production. In-feed antibiotics are commonly used to control the impact of clinical diseases on production parameters and economics. The objective of this trial was to compare the effect of four levels of prophylactic treatment on total weight gain and feed conversion rate (FCR) in a group of PRRS negative grow-finish pigs, where Enzootic Pneumonia and PPE had been previously diagnosed. Most cases of morbidity and mortality in this herd were diagnosed, by postmortem examination and histopathology, as concurrent enteric and respiratory diseases due to acute hemorrhagic ileitis and enzootic pneumonia, despite the use of Tylosin at a dose of 55g/ton.

## Materials and Methods

Eighty crossbred (Dekalb 45 female X NSR American Landrace boar) pigs within a continuous flow production system which had historical 3.5% mortality, and a 2.7 FCR were used. The pigs were randomized by weight to one of four treatment groups with 20 pigs per group (Fig. 1). A previous selection based on minimum weight variation, one day before grouping was performed. Pigs were fed a soybean-corn-based diet that followed Kansas State University recommended nutritional values. Starting weights ranged from 21 to 24kg and expected market weight ranged from 95 to 100kg. Initial and final body weights were recorded

using an electronic scale. Clinical signs of diarrhea and pneumonia were observed and registered for pigs as a group.

Data was processed by analysis of variance at the 95% of confidence level.

## Results and Discussion

After 84.7 days on feed, the results for total average gain in Kg and FCR were:

Figure 1. Treatment groups, gain in kg, and FCR

Treated Groups	Antibiotic dose	Total gain (kg)	Average FCR
Group 1	Tylosin 110g/Ton	80.56 <sup>a,c</sup>	2.42
Group 2	Tylosin 55g/Ton	78.27 <sup>b</sup>	2.53
Group 3	Valnemulin 75g/Ton	81.51 <sup>a</sup>	2.37
Group 4	Valnemulin 37.5g/Ton	79.90 <sup>c</sup>	2.41

\* a different letter denotes a statistically significant difference ( $p < 0.000016$ ).

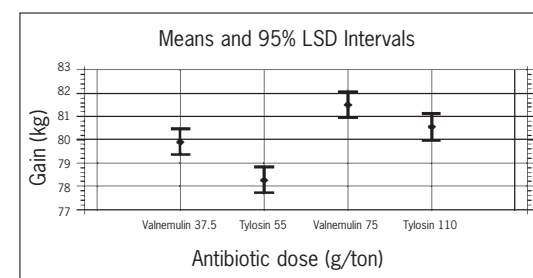
In Group 3 Valnemulin 75g/ton had the highest weight gain and the best FCR ( $p \leq 0.000016$ ). There was no statistical difference between Group 1 Tylosin 110g/ton and Group 4 Valnemulin 37.5g/ton.

None of the animals died during the trial. Clinical signs of diarrhea were observed in 3 of 80 pigs (3.75%) and clinical signs of pneumonia were observed in 2 of 80 pigs (2.5%).

## Conclusion

For this field trial, these results indicate that Valnemulin 75g/ton was the most effective dose, however Valnemulin 37.5g/ton can be considered as a medium point for a cost-effective ratio, to obtain an acceptable production and health outcome, based on production results and clinical history.

Figure 2. Total weight gain per pig/ dose (g/ton)



References.  
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S. Kesdangakonwut and others

## A COMBINATION OF VALNEMULIN AND DOXYCYCLINE FOR CONTROLLING BACTERIAL RESPIRATORY DISEASES IN WEANLING PIGS IN A PRRSV-POSITIVE HERD: A FIELD TRIAL IN THAILAND

A field trial in a PRRSV- positive herd showed that ECONOR (75ppm) in combination with doxycycline (250ppm) was more effective in the control of respiratory bacterial infections than the combination of Lincospectin (100ppm) and chlortetracycline (300ppm).

Parameter	Lincospectin/CTC	ECONOR / Doxycycline	Improvement
Average daily gain (g)	514.68 <sup>b</sup>	731.92 <sup>a</sup>	42%
FCR	2.26	1.63	28%
Gross lung score (%)	8.33	4.66	
Histological lung score (0-3)	2.67	2.33	
Mycoplasma ELISA-positive (%) start/end of trial	50/83.3	50/0	

### KEY FACTS

- The combination of ECONOR and doxycycline is very effective in the treatment of complicated respiratory bacterial infections caused by *M. hyopneumoniae* and other bacterial respiratory pathogens such as *B. bronchiseptica*, *P. multocida* and *S. suis*.
- ECONOR and doxycycline showed a superior efficacy in comparison to Lincospectin and chlortetracycline.
- The lower number of bacterial pathogens in nasal and tracheal swabs confirm the excellent effect of ECONOR + doxycycline. These bacteriological results correspond well with the lower severity of the lung lesions in the ECONOR/doxycycline group.
- The results strongly support the therapeutic use of ECONOR in combination with doxycycline for the treatment of mycoplasmal/bacterial respiratory diseases in swine.

# A combination of valnemulin (ECONOR®) and doxycycline for controlling bacterial respiratory diseases in weanling pigs in a PRRSV-positive herd: A field trial in Thailand

S. Kesdangsakonwut<sup>1</sup>, S. Sukchai<sup>2</sup>, M. Makhanon<sup>2</sup>, R. Thanawongnuwech<sup>1</sup>  
 Veterinary Pathology, Chulalongkorn University<sup>1</sup> and Novartis (Thailand) Limited<sup>2</sup>, Bangkok, Thailand.  
**Keywords:** PRRSV, bacteria, respiratory, valnemulin, doxycycline, pigs.

## Introduction and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important causative agent for severe respiratory disease in nursery pigs. PRRSV commonly induces severe secondary bacterial infections such as *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumonia* (*APP*), or *Streptococcus suis* etc.<sup>3,5</sup> Medicated feed was one of many tools using for improving the productive performance in the PRRSV-infected farms<sup>6</sup>. Ceftiofur hydrochloride injection yielded satisfactory results for minimizing disease associated with PRRSV and *S. suis* coinfection<sup>1</sup>. In addition, *M. hyo* vaccination at a proper time might reduce the severity of PRRSV-induced pneumonia<sup>2</sup>. Our objective was to evaluate the efficacy of a combination of valnemulin (ECONOR®) and doxycycline for controlling the respiratory diseases in the weanling pigs in a PRRSV-positive farm in Thailand.

## Materials and Methods

A fattening pig farm with the previous problems of PRRSV determined by the farm history was selected. Forty, 10-week-old, cross-bred pigs were assigned into 2 groups of 20 pigs each. Treatment I was given medicated feed with a combination of valnemulin (75 ppm) and doxycyclin (250 ppm). Treatment II was given medicated feed with lincospectin (100 ppm) and chlortetracycline (300 ppm). Each pig was tagged and treated with the same routine management of the farm for 3 weeks. All parameters such as weighting, bacterial culture (nasal and tracheal cultures), serological tests for

Mycoplasmal and PRRSV antibodies were done both at the beginning and finishing dates of the trial. Nested PCR for PRRSV detection using individual serum was performed at the end of the trial<sup>4</sup>. Percentage of dead loss and cost of medications and treatments using through the three weeks treatments of each group were recorded. Six pigs from each group were randomly selected for necropsy and lungs were evaluated using a scoring system<sup>1</sup>. Lung tissues were taken for histopathological examination and estimated score (0-3) of the severity of the interstitial pneumonia was also recorded<sup>1</sup>. Other significant lesions present at necropsy were also recorded.

## Results and Discussion

The production parameters correlating with the clinical performance (data not shown) demonstrated that Treatment I yielded better ADG and FCR than those of the Treatment II (Table1). Cranioventral pneumonia was predominantly seen in both group, but worse lesions were seen in the Treatment II concurrent with chronic pleuritis (33.33%) and swollen of tracheo-bronchial lymph nodes. Microscopic lesions characterized by thickening of alveolar wall and peribronchiolar lymphoid hyperplasia with suppurative bronchopneumonia indicated the presence of concurrent infection between PRRSV, mycoplasma and possibly other bacteria. PRRSV definitely played a major role in the respiratory complex in this farm since the PCR was able to demonstrate the presence of the virus in the circulation. The presence of

higher numbers of bacterial culture at necropsy was correlated well with the severity of the lung lesions. However, both treatments appeared to have an indirect effect on those secondary bacterial infections, but possibly against *M. hyo* directly. The results from this study demonstrated that the combination of the antimicrobials in Treatment I was better in minimizing the negative effects of secondary bacterial infection in this PRRSV-positive farm than that of the Treatment II. The particular combination of valnemulin (ECONOR®) and doxycycline may directly reduce the clinical signs and lesions of *M. hyo* infection as indicated by no seroconversion of *M. hyo* in the Treatment I at the end of the trial. The pigs in the Treatment I, therefore, had greater production parameters.

Table 1. Production parameter (LSMEAN ± SEM, n=20) and lung scores (n=6)

Parameter	Treatment I	Treatment II
ADG (Gm/day)	731.92 ± 45.20 <sup>a</sup>	514.68 ± 45.20 <sup>b</sup>
FCR	1.63 ± 0.41	2.26 ± 0.42
Lung score (%)	4.66 ± 6.12	8.33 ± 6.12
Histo. score (0-3)	2.33 ± 0.52	2.67 ± 0.82

<sup>a,b</sup> Values in the same row with different characters are statistically different (p≤0.05)

Table 2. PCR (n=6) and serological results (n=20)

% Positive	PRRSV PCR		PRRSV ELISA		Mycoplasma ELISA	
	Begin	Finish	Begin	Finish	Begin	Finish
Treatment I	ND	83.33	85	83.33	50	0
Treatment II	ND	66.67	95	100	50	83.33

ND: not determined

Table 3. Bacteriology results from nasal (beginning date) and tracheal (finishing date) swabs

	Treatment I		Treatment II	
	Nasal	Trachea	Nasal	Trachea
<i>α-Streptococcus sp.</i>	70	0	60	16.67
<i>Haemophilus sp.</i>	5	0	5	0
<i>B. bronchiseptica</i>	0	83.33	0	100
<i>P. multocida</i>	0	0	0	16.67
<i>A. pleuropneumoniae</i>	15	66.67	30	50

## Acknowledgements

This study was funded by Novartis (Thailand) Limited.

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L. Fodor and others

## 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<sub>50</sub> and MIC<sub>90</sub> of the tested antibiotics for *P. multocida*, *S. suis*, *B. bronchiseptica* and *A. pleuropneumoniae* (µg/ml)

	<i>P. multocida</i>			<i>S. suis</i>		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Val</b>	1.0-4.0	2.0	4.0	0.015-0.125	0.03	0.03
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
	<i>B. bronchiseptica</i>			<i>A. pleuropneumoniae</i>		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Val</b>	8.0-16.0	8.0	16.0	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 valnemulin and doxycycline alone and in combination

	<i>P. multocida</i>		<i>S. suis</i>		<i>B. bronchiseptica</i>		<i>A. pleuropneumoniae</i>	
	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	-
<b>Val alone</b>	1.741	-	0.023	-	11.31	-	2.143	-
Comb. Dox	0.016	7.8 x	0.02	9.5 x	0.010	8.8 x	0.143	3.0 x
<b>Comb. Val</b>	0.435	4.0 x	0.011	2.1 x	4.287	2.6 x	0.870	2.5 x

### KEY FACTS

- Valnemulin is demonstrated in vitro to be more active against *S. suis* than a range of competitor products – tylosin, lincomycin, tilmicosin, CTC, doxycycline.
- The trial data prove the synergistic activity of valnemulin 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 2.1-9.5 times lower MIC values.
- The new in vitro results are consistent with previously generated data demonstrating the synergism between valnemulin and tetracyclines.
- The combination of ECONOR 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

L. Fodor<sup>1</sup>, L. Stipkovits<sup>2</sup> and U. Klein<sup>3</sup>

Szent Istvan University, Veterinary Faculty, Department of Microbiology and Epidemiology, Budapest, Hungary<sup>1</sup>, Veterinary Research Institute, Hungarian Academia of Sciences, Budapest, Hungary<sup>2</sup>, Novartis Animal Health, Basel, Switzerland<sup>3</sup>.

## 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 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 cellophane 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<sub>50</sub> and MIC<sub>90</sub> 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<sub>50</sub> and MIC<sub>90</sub> of tested antibiotics for *P. multocida* and *S. suis*

	<i>P. multocida</i>			<i>S. suis</i>		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
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<sub>50</sub> and MIC<sub>90</sub> of tested antibiotics for *B. bronchiseptica* and *A. pleuropneumoniae*

	<i>B. bronchiseptica</i>			<i>A. pleuropneumoniae</i>		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
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

	<i>P. multocida</i>		<i>S. suis</i>		<i>B. bronchiseptica</i>		<i>A. pleuropneumoniae</i>	
	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

L. Stipkovits and others

## SENSITIVITY TESTING OF MYCOPLASMA PATHOGENS TO VALNEMULIN (ECONOR®) AND OTHER ANTIMICROBIALS

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

Table 1: MIC range, MIC<sub>50</sub> and MIC<sub>90</sub> of the tested antibiotics for *M. hyopneumoniae*/*M. hyosynoviae*/*M. hyorhinae* (µg/ml)

	<i>M. hyopneumoniae</i>			<i>M. hyosynoviae</i>			<i>M. hyorhinae</i>		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Val</b>	0.06-1.0	0.06	0.5	0.015-0.125	0.06	0.125	0.06-0.5	0.06	0.25
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 valnemulin and doxycycline alone and in combination

	<i>M. hyopneumoniae</i>		<i>M. hyosynoviae</i>		<i>M. hyorhinae</i>	
	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor	MIC (av.)	Syn. factor
Dox alone	5.169	-	1.101	-	0.933	-
<b>Val alone</b>	<b>0.120</b>	<b>-</b>	<b>0.069</b>	<b>-</b>	<b>0.091</b>	<b>-</b>
Comb. Dox	0.466	11.1 x	0.116	9.5 x	0.076	12.3 x
<b>Comb. Val</b>	<b>0.101</b>	<b>1.2 x</b>	<b>0.020</b>	<b>3.5 x</b>	<b>0.041</b>	<b>2.2 x</b>

### KEY FACTS

- The Mycoplasma strains tested showed the highest sensitivity to valnemulin.
- The MIC<sub>90</sub> values of doxycycline, tylosin, lincomycin, chlortetracycline and tilmicosin were between 4-256 times higher than those of valnemulin.
- The MIC data confirm the exceptional potency of valnemulin against *Mycoplasma hyorhinae* and *Mycoplasma hyosynoviae*.
- Valnemulin combined with doxycycline possesses a synergistic activity.
- The synergistic activity of valnemulin and doxycycline against the *M. hyopneumoniae*, *M. hyorhinae* and *M. hyosynoviae* strains resulted in 1.2-12.3 times lower MIC values.
- The new in vitro results are consistent with previously generated Mycoplasma-related data and the clinical response to ECONOR observed in the field and in many in vivo studies.

# Sensitivity testing of Mycoplasma pathogens to antimicrobials

L. Stipkovits<sup>1</sup>, J. Biro<sup>1</sup>, S. Szathmary<sup>1</sup> and U. Klein<sup>2</sup>

<sup>1</sup>Institute of Veterinary Research, Hungarian Academy of Sciences, Budapest, Hungary <sup>1</sup>, Novartis Animal Health, Basel, Switzerland <sup>2</sup>.

## Introduction

Mycoplasma pneumoniae 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 450 nm, 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 cellophane 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<sub>50</sub> and MIC<sub>90</sub> 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) II. 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<sub>50</sub> and MIC<sub>90</sub> of the tested antibiotics for M. hyopneumoniae/hyosynoviae/hyorhinis

	M. hyopneumoniae			M. hyosynoviae			M. hyorhinis		
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
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
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
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. MIC values of antibiotics alone/in combinations

	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	-
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



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\*Swine Dysentery, Ileitis, Spirochaetal Colitis \*\*Report of the EU's Committee for Veterinary Medicinal Products on Antibiotic Resistance in the EU associated with Therapeutic use of Veterinary Medicines (1999)