SPONSORS' PAPERS

VALNEMULIN – FOR THE PREVENTION AND TREATMENT OF COLITIS AND ILEITIS

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Summary

Valnemulin (Econor – Novartis) was approved originally in 1999 by the European Union (EU) for the prevention and treatment of swine dysentery caused by Brachyspira hyodysenteriae and enzootic pneumonia caused by Mycoplasma hyopneumoniae. Recent work has showed that valnemulin is also highly active against B. pilosicoli, a spirochaete with many similarities to B. hyodysenteriae, which causes porcine spirochaetal diarrhoea or colitis, a milder form of diarrhoea in grower pigs. It has also been shown to have a high level of intracellular activity against Lawsonia intracellularis, the cause of porcine proliferative enteropathy or ileitis. The paper reviews the clinical trial work, both artificial challenge models and field studies, that enabled valnemulin to be recently approved for the prevention of colitis and treatment of ileitis in the EU.

Introduction

Valnemulin (Econor – Novartis) has recently been approved by the European Union (EU) for the prevention of colitis (porcine colonic spirochaetosis - PCS) caused by *Brachyspira pilosicoli* and treatment of ileitis (porcine proliferative enteropathy) caused by *Lawsonia intracellularis.* These claims are added to the existing enteric claim of prevention (25ppm valnemulin) and treatment (75ppm valnemulin) of swine dysentery caused by *B. hyodysenteriae*, making it the only EU-approved medicated feed premix for all of these three enteric diseases. These infections are commonly seen, either alone or mixed together, and account for the majority of diarrhoeas involving growing pigs. The purpose of this paper is to review the interaction of these infections and examine the work presented for the new valnemulin claims for colitis and ileitis.

'Colitis' in the growing pig

The condition, commonly referred to as 'colitis,' can be caused by a number of infectious agents but, clinically, usually affects pigs of 6-12 weeks of age with a non-specific grey to brown diarrhoea, frequently without blood but often with mucus, affecting up to 50% of the animals. There is usually a low mortality of 2-5%, but also a steady reduction in performance parameters, such as growth rate and feed conversion efficiency, of approximately 10-20%. Complications can arise, such as rectal prolapse, which may subsequently develop into rectal stenosis and stricture, more normally seen in the finishing house.

In a survey carried out on 85 pig units in Scotland with grower (15-50kg bodyweight) diarrhoea problems (Thomson *et al*, 1998), a number of potentially pathogenic organisms were isolated or demonstrated histologically in the case of *L. intracellularis*.

Table 1 - Infectious causes of 'colitis' and their incidence (Thomson *et al*, 1998)

Organism	Single	Mixed	Total	Units affected (%)	Identifications (%)
B. pilosicoli	21	23	44	52	39
Atypical Brachyspira	7	2	9	11	8
B. hyodysenteriae	6	3	9	11	8
L. intracellularis	3	10	13	15	12
Salmonella spp	4	8	12	14	11
Y. pseudotuberculosis	4	13	17	20	15
E. coli	1	5	6	7	5
C. perfringens	0	2	2	2	2

B. pilosicoli, other brachyspiras including *B. hyodysenteriae* and *L. intracellularis* were found in 67% of the species identifications either as single or mixed infections; but were found on 79% of the units examined. These organisms play an important role in diarrhoea in this stage, in contrast to *Escherichia coli*, which is more significant in the younger pig, especially after weaning. It is a little surprising that the identification of *L. intracellularis* was so low at 15%, as Mortimer *et al* (2000) had shown serologically that 95% of UK and Irish herds were infected. Ileitis may not be such a widespread clinical problem (15% of units); but brachyspira infections are more so, affecting 74% of the units.

Porcine colonic spirochaetosis – colitis – *B. pilosicoli*

Over recent years, much work has been carried out on classifying the various brachyspira species, both biochemically and genetically, especially using a random amplified polymorphic (RAPD) technique and they are currently classed in 4 major groups.

Table 2 - Brachyspira classification (Fellstrom *et al*, 2003)

Group	**Brachyspira species**
I	*B. hyodysenteriae*
II	*B. intermedia*
IIIa	*B. murdochii*
IIIbc	*B. innocens*
IV	*B. pilosicoli*

Thomson *et al* (2003) compared the pathogenicity of *B. hyodysenteriae* isolates of pathogenic and mild disease-causing ability with *B. pilosicoli* in artificial challenge studies. The pigs were scored clinically during the study and weighed at the beginning and end and the average daily gain (ADG) calculated. Necropsies were carried out 14-15 days post-infection and the gross and histopathological lesions scored.

Table 3 - Comparison of *B. hyodysenteriae* and *B. pilosicoli* infections in pigs (Thomson *et al*, 2003)

Group	**Feature**	**Clinical score (%)**	**Pathological score at necropsy (%)**	**ADG (g)**
Control	Uninfected	0	1	810 (-)
B. hyodysenteriae	Pathogenic form	29	45	590 (-27%)
B. hyodysenteriae	Mild form	8	16	740 (-9%)
B. pilosicoli	-	10	19	710 (-12%)

B. pilosicoli usually shows a more moderate pathogenic effect in comparison with a pathogenic strain of *B. hyodysenteriae*, but a similar effect to a mild form of *B. hyodysenteriae.* They also showed in similar trials that *B. murdochii* and *B. intermedia* reference strains had almost no pathogenic effect in pigs.

Apart from its pathogenicity, *B. pilosicoli* is more ubiquitous than *B. hyodysenteriae* and less host specific. It can colonise birds, rodents, man and dogs. Otherwise it colonises the colon in a similar way, attaching end-on to the mucosal epithelial cells (carpet-pile effect) and may invade the crypts and cause a mild to moderate inflammatory response. The organism may also colonise damaged epithelia especially associated with porcine proliferative enteropathy (ileitis), which can spread into the colon and caecum from the ileum.

B. pilosicoli has been shown to be very sensitive to valnemulin (Ripley, 1998) in a minimal inhibitory concentration (MIC) study using agar plate dilution tests.

Table 4 - Comparison of various antimicrobials MIC against 12 UK isolates of *B. pilosicoli* (Ripley, 1998)

Antimicrobial	MIC50 (µg/ml)	Range (µg/ml)
Valnemulin	0.018	<0.015-0.125
Tiamulin	0.067	0.031-0.5
Lincomycin	4.32	0.25-12.5
Tylosin	31.6	10->200
Dimetridazole	0.53	0.062-1.0

In a further MIC study (Kinyon *et al*, 2002), looking at 25 North American isolates using a plate dilution test, valnemulin was again shown to be highly active against *B. pilosicoli*. There was much more marked resistance to lincomycin and tylosin.

Table 5 - Comparison of various antimicrobials MICs against 25 N. American isolates of *B. pilosicoli* (Kinyon *et al*, 2002)

Antimicrobial	MIC50 (µg/ml)	MIC90 (µg/ml)	Range (µg/ml)
Valnemulin	0.06	0.5	0.03-2.0
Tiamulin	0.125	1.0	0.06-8.0
Lincomycin	32	64	4.0->128
Tylosin	>512	>512	<16->512
Carbadox	0.06	0.06	0.03-0.125

A concentration of valnemulin 1.68µg/ml was achieved in the colon after feeding 75ppm in feed, approximately the upper limit of the range of MICs found in the N. American isolates. At 25ppm, this concentration could be extrapolated to 0.56µg/ml, approximately the MIC 90 for the N. American isolates and the range tested in the UK.

Artificial challenge studies – *B. pilosicoli*

Ripley (1998) reported on an artificial challenge study where two groups of nine pigs were challenged orally with cultures of *B. pilosicoli*. One group received valnemulin at 25ppm and this was given for 4 days before infection and both groups were necropsied on day 14 after infection.

There were few clinical signs during the trial, with only one untreated infected control and only a low-grade infection in some of the remainder.

Table 6 - First prevention of colitis, artificial challenge study with *B. pilosicoli* (Ripley, 1998)

	Untreated infected control	**Valnemulin 25ppm**
No. of pigs	9	9
No. with diarrhoea	1	0
No. with gross lesions	2	0
No. histological lesions	6	0
No. with spirochaetes	2	0

No lesions or evidence of infection were observed in the valnemulin 25ppm prevention group; but it was a very mild challenge.

In a second study (Morgan *et al*, 2002), adjustments were made to the model to increase the incidence of the infection by using a high soya/wheat diet. Young pigs of 4-6 weeks were used in the study and allocated to two groups with six replicates of six pigs. They were acclimatised to the diet for one week and then challenged on two consecutive days with cultures of *B. pilosicoli* isolated from a field infection in the UK. Feed containing valnemulin at 25ppm (to give a dose of 1.25mg/kg bodyweight) was given two days after infection and continued for 27 days. Faecal samples were taken thrice weekly and the pigs were scored for the presence of diarrhoea and their weight gain and feed intake were recorded.

Table 7 - Second prevention of colitis, artificial challenge study with *B. pilosicoli* (Morgan *et al*, 2002)

	Untreated infected control	**Valnemulin 25ppm**	**Improvement (%)**
Colonisation	32/35 (91%)	1/36* (3%)	97
No. with diarrhoea	11/35	10/36	-
Days with diarrhoea	111	31*	72
Ave. diarrhoea score	0.54	0.19*	65
ADG (g)	470	610*	30
FCE	1.75	1.49*	15

(Key: * p =<0.05)

Although the diarrhoea was not completely halted in the treated group, it was significantly reduced. They were quite young pigs and, as such, are prone to diarrhoea. There was a significant improvement in growth rates and feed conversion efficiency; but the medication almost completely inhibited the isolation of *B. pilosicoli* from faeces (97%), demonstrating that valnemulin at 25 ppm is very effective in preventing colitis.

In a third study (Duhamel *et al*, 2002), four week-old pigs were divided into two groups of six pens each containing five pigs. On day 0 they were inoculated orally with *B. pilosicoli*. On day 13, when approximately 15% of the pigs or more showed signs of diarrhoea and 26.7% of the pigs were excreting *B. pilosicoli*, one group received medicated feed with valnemulin at 25ppm and the other remained untreated. Necropsies were carried out on day 27.

Table 8 - Treatment of colitis, artificial challenge study with *B. pilosicoli* (Duhamel *et al*, 2002)

Colonisation *B. pilosicoli*	**Untreated infected control**	**Valnemulin 25ppm**
Day 13 (faeces)	26.7	26.7
Day 20 (faeces)	20	0
Day 27 (necropsy)	41.4	0

There were significant reductions in the incidence of diarrhoea in the valnemulin group and also in histopathological lesions in the colon in comparison with the untreated infected controls. The lesions observed suggested that by day 27 most pigs had recovered from the acute infection. There were no gross lesions

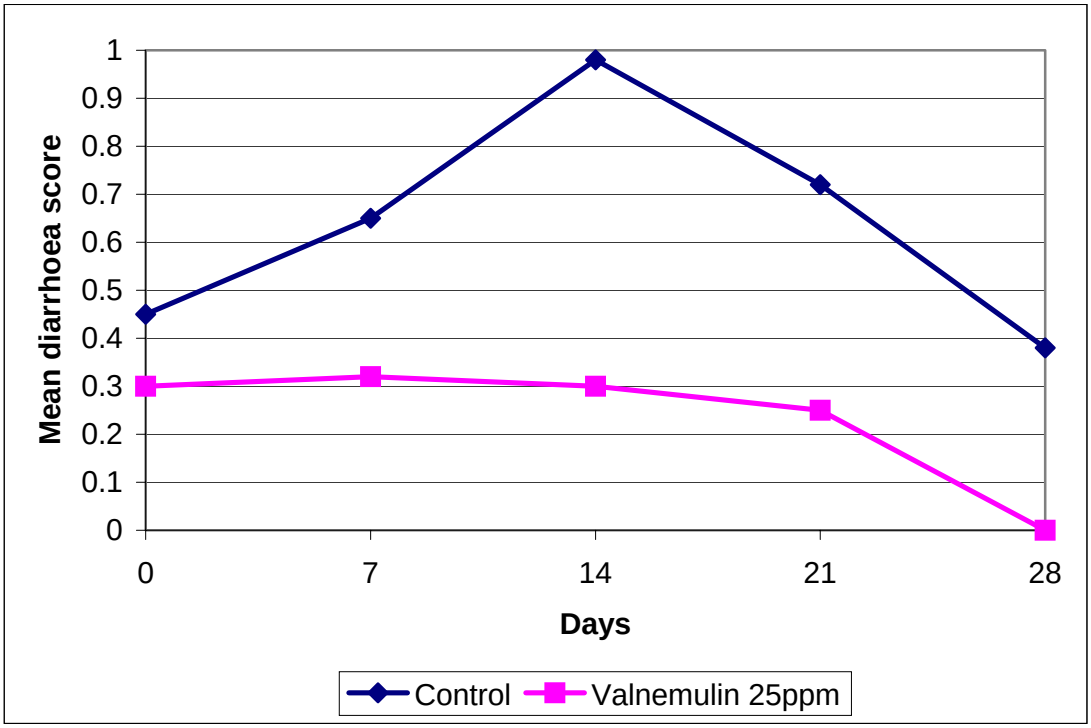
seen at necropsy and there were no significant differences in growth rate and feed conversion efficiency. Valnemulin at 25ppm reduced clinical signs of colitis and also eliminated the spirochaetes in what can be considered a treatment study, as clinical signs had already developed prior to the commencement of treatment.

Field studies – *B. pilosicoli*

In a UK field study (Glossop *et al*, 2000), valnemulin at 25ppm was tested against a naturally occurring *B. pilosicoli* infection, uncomplicated by other spirochaetes and ileitis. Piglets were born outdoors and brought into straw-kenneled yards at weaning at about 4 weeks of age. Usually 2-4 weeks later they broke down with diarrhoea attributed to the *B. pilosicoli* infection.

One hundred and twenty pigs were randomised into 6 pens of 20 pigs and divided into two treatment groups, an untreated control and the valnemulin 25ppm group. Diarrhoea had already started in a few pigs (5%) at the start of the trial and rapidly developed in the untreated group (30%); but remained low in the valnemulin group. The trial lasted for 28days.

Graph 1 -Mean diarrhoea scores for the untreated control and valnemulin 25ppm groups during the trial



A good prevention of diarrhoea was achieved in the valnemulin group. A peak seemed to occur in the untreated control at about 14 days and then natural recovery developed upto day 28, supporting the observations of Duhamel *et al* (2002) that natural recovery starts to take place during this time.

Table 9 - Prevention of colitis with valnemulin 25ppm field trial results

Results day 0-28	**Untreated control**	**Valnemulin 25ppm**
ADG (g)	365	429*
FCE	2.167	2.026
Mean cumulative condition score	3.1	1.5*
Mean cumulative faeces score	6.5	3.0*

(Key: * p = <0.05)

Conclusions - colitis

Valnemulin at 25ppm was highly effective in preventing the clinical signs of colitis as judged by reduced clinical signs and improvement in performance of both weight gains and feed conversion efficiency. In the artificial challenge studies, there was a 97-100% reduction in *B. pilosicoli* shedding and colonisation. In the field, valnemulin 25ppm was also highly effective in the prevention of colitis.

Porcine proliferative enteropathy – ileitis – *Lawsonia intracellularis*

Ileitis is an infectious condition, caused by *L. intracellularis*, and characterised by proliferative changes in the epithelium of both the small and large intestinal mucosa (Taylor 1999). Related conditions are necrotic enteritis, regional ileitis and proliferative haemorrhagic enteropathy or 'bloody gut.'

Infection is by the oral route usually due to faecal contamination and is especially seen in continuous flow systems with poor hygiene. The organism, which can survive outside the host for 14 days, is ingested, quickly invades and multiplies inside the epithelial cell, eventually leading to its rupture and destruction. Bacteria are shed and can invade other cells or may pass out of the host in the faeces. Early changes in the mucosa are cell hyperplasia especially in the terminal ileum, leading to destruction of the villus apex and the leakage of blood. Other bacteria appear to exacerbate the lesions, e.g. *Bacteroides vulgatus*, *E. coli* and also *B. pilosicoli*. Mucosal changes appear 8-10 days after infection and peak at about 21 days when resolution starts to take place as immunity develops. The lesions can spread up the ileum to the jejunum and down into the

caecum and proximal colon. Shedding of *L. intracellularis* starts at about 13 days post-infection and may last for up to 10 weeks.

Clinically, the disease normally affects pigs 3-4 weeks after weaning, but may occur even in adults. Medication can alter the pattern of infection in herds, delaying it to the finishing period. In most cases there is diarrhoea, reduction in growth and feed conversion efficiency and, occasionally, acute deaths due to the haemorrhagic form often in heavier fattening pigs.

The organism cannot be grown on agar and is cultured in rat enterocyte tissue cultures. An ingenious method of testing the sensitivity of an antimicrobial was devised by McOrist *et al* (1995) by infecting cell cultures and growing them in the presence of varying concentrations of antimicrobials. Where 99% of the growth was inhibited, the 'intracellular MIC' was determined. Burch (2003) demonstrated that the growth inhibition curves of the organism gave a good direct relationship to the concentration of the antimicrobial in the terminal ileum.

The intracellular MIC for valnemulin against *L. intracellularis* was reported as <2.0µg/ml McOrist *et al*, 1998). In fact, the lower limits of inhibition were not determined, as 2.0µg/ml was the lowest limit tested and caused almost 100% inhibition (McOrist, 1994 - unpublished data).

Artificial challenge studies – *L. intracellularis*

McOrist *et al* (1998) carried out a valnemulin dose-titration study to look at the prevention and treatment of disease produced by an artificial oral challenge with cell cultures infected with *L. intracellularis*. This model usually induced lesions of the chronic form of the disease and typical clinical signs of diarrhoea but no mortality, about 2-3 weeks after infection. In the prevention study, valnemulin was administered in the feed at 0, 25, 37.5 and 50ppm two days before infection and in the treatment part at 75 and 125ppm seven days after infection until day 21. There were seven pigs per treatment group. The pigs were then necropsied and the intestines examined for gross and histopathological lesions.

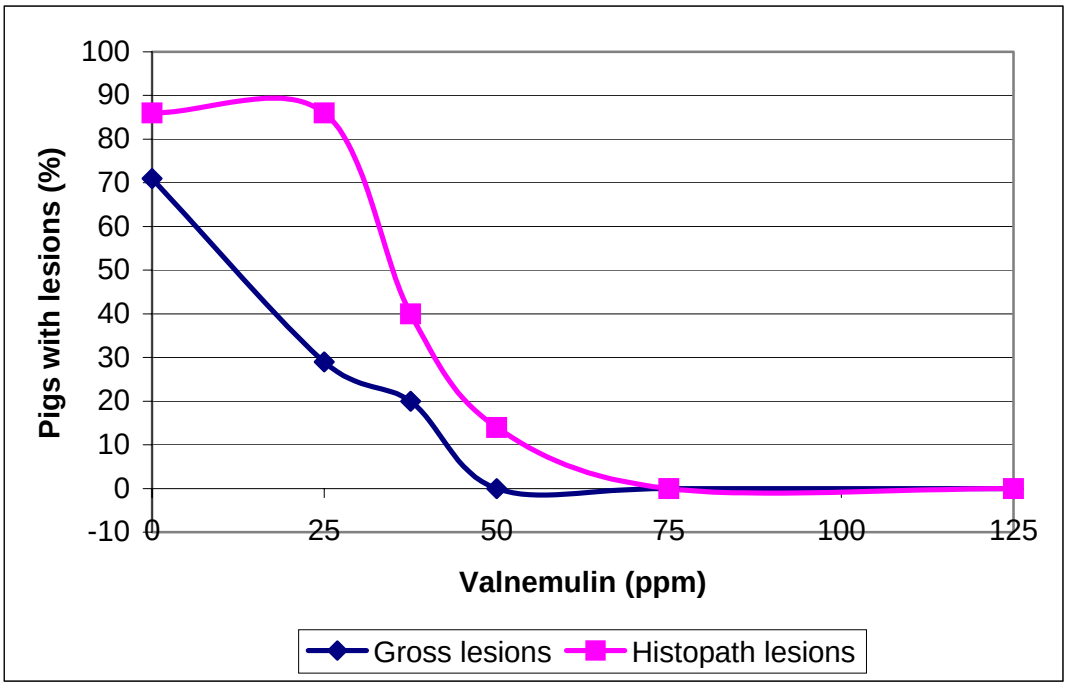
Table 10 - Results of valnemulin dose-titration study using an artificial challenge of *L. intracellularis* in cell culture (McOrist *et al*, 1998)

Treatment	No. with gross lesions (%)	No. with histopathological lesions (%)	Weight gain (kg)	FCE
Untreated infected control	71	86	4.1	2.00
Valnemulin 25ppm P	29	86	6.0	1.47
Valnemulin 37.5ppm P	20	29	5.4	1.52
Valnemulin 50ppm P	0	14	5.5	1.56
Valnemulin 75ppm T	0	0	5.2	1.58
Valnemulin 125ppm T	0	0	5.6	1.50

Key: P = prevention; T = treatment

Marked improvements in performance figures were seen, even at the 25ppm valnemulin level, for both growth and FCE in comparison with untreated controls. There were reductions in gross lesions at all levels; although they were completely prevented at 50ppm and treated from 75ppm and above. Histopathological lesions could still be detected at 50ppm; but they were eliminated at 75ppm, the recently approved treatment level.

Graph 2 - Effect of varying levels of valnemulin in the feed on gross and histopathological lesions of ileitis



In a second study (Winkleman *et al*, 2000a), used a much more severe challenge model, which produced a high mortality (32%). Valnemulin at 0, 25, 37.5, 50ppm was tested against a positive control of tylosin at 110ppm in a prevention study. There were five pens of five pigs per treatment group. Medicated feed was given 5 days before infection and was continued for 21 days after infection. Pigs were infected on two consecutive days with a ground up mucosal homogenate from a previously infected pig. Mortality, diarrhoea scores and growth rates were reported.

Table 11 - Results of valnemulin dose-titration study using an artificial challenge of *L. intracellularis* lesion homogenate for the prevention of ileitis (Winkleman *et al*, 2000a)

Treatment	**Mortality (%)**	**Individual diarrhoea score day 14 (adj)**	**ADG (g) day 0-21**
Untreated infected control	32	1.4	40
Valnemulin 25ppm	0*	0.5*	250*
Valnemulin 37.5ppm	0*	0.3*	270*
Valnemulin 50ppm	0*	0.2*	330**
Tylosin 110ppm	0*	0.5*	250*

Key: * p = <0.05; ** p = <0.05*; adj = score adjusted to 0 for normal

There was very high mortality in the untreated infected controls, of 32%. This challenge model is very severe and does not mirror the field situation. However there was no mortality in any of the treatment groups, diarrhoea was reduced and growth rate dramatically increased, the valnemulin 50ppm being significantly better than the tylosin 110ppm group. Valnemulin at 25ppm equalled the positive control tylosin 110ppm results in preventing ileitis.

A third study (Winkleman *et al*, 2000b) used valnemulin at 0, 25 and 50ppm for the prevention of ileitis, again using the ground mucosal homogenate. The pigs were quite young at 5-6 weeks of age and there were 3-4 pens of 10 pigs per group. Treatment commenced at the time of infection and continued for 21 days when the pigs were necropsied and the lesions scored and measured. The pigs were examined clinically and faeces scored during the trial and weight gains and feed conversion efficiency calculated.

Table 12 - Results of an artificial challenge study with 25 and 50ppm valnemulin for the prevention of ileitis (Winkleman *et al,* 2000b)

Treatment	**Mortality (%)**	**Lesion length (cm)**	**Diarrhoea score (day 20-0)**	**ADG (g)**	**FCE**
Untreated infected control	6.7	141.3 (-)	1.33	160	4.54
Valnemulin 25ppm	2.5	94.6* (-33%)	0.54*	290*(81%)	3.34
Valnemulin 50ppm	0	72.0* (-49%)	0.41*	360*(125%)	3.24

Key: *p = <0.05

The mortality rate in unmedicated controls was much lower in this study, but reduced completely in the 50ppm treatment group. The lesions were very severe and extensive at 141cms, involving the jejunum, ileum, caecum and colon. Valnemulin at 50ppm reduced them by 49%. Growth rate and feed conversion were also better in the valnemulin group; suggesting a dose response effect similar to the earlier trial but not elimination.

Field trials – *L. intracellularis*

Haugegaard *et al* (2000) described a field trial in Denmark where 120 pigs on a unit where the presence of ileitis was proven by polymerase chain reaction (PCR) testing. Pigs were allocated to two groups of three pens of 20 pigs. When mild to moderate diarrhoea occurred in 20% of the animals one group received

valnemulin at 75ppm for 10 days and the other remained untreated. The pigs were observed for another 10 days. The pigs were examined during the trial and scored for clinical appearance and diarrhoea. They were weighed on day 0, 10 and day 19 and their feed intake recorded.

Table 13 - Results of a field trial with 75ppm valnemulin for the treatment of ileitis (Haugegaard *et al*, 2000)

	Untreated control	**Valnemulin 75ppm**
Ave. diarrhoea score day 0	0.71	0.82
Day 5	1.2	0.67
Day 10	0.9	0.67
Cumulative score day 0-10	4.2	2.6* (-38%)
ADG (g) day 0-10	506	658* (12%)
FCE day 0-10	1.78	1.48* (-17%)

There was a good clinical response and the diarrhoea score reduced in the valnemulin treated group, while rising in the untreated pigs. Weight gain and FCE were also substantially improved in the valnemulin treated group.

In a second study in the USA (Holck *et al*, 2002), 120 cross-bred pigs approximately 24 weeks of age (62-103kg bodyweight), experiencing an acute outbreak of ileitis, were taken to a research facility for the trial. They were allocated to two treatment groups in 12 pens of five pigs each. One group was treated with valnemulin at 75ppm in feed for 10 days and the other acting as the untreated control. The trial lasted 28 days.

Table 14 - Results of a field trial with valnemulin 75ppm for the treatment of ileitis (Holck *et al,* 2002)

	Untreated control	**Valnemulin 75ppm**
Mortality (%)	5.0	1.7
Ave. diarrhoea score day 0 (adj)	1.5	1.6
Day 5	1.1	1.4
Day 10	0.8	0.4
ADG (g) day 0-10	400	630* (58%)
FCE day 0-10	4.56	3.12* (-32%)

Key: * p = <0.05; adj = score adjusted from 1 for normal to 0 for normal

Valnemulin medication reduced mortality. There was a trend for diarrhoea score reduction during the trial but the valnemulin was faster by day 10. The improvements in ADG and FCE were exceptional. The valnemulin dose was very

low at 1.58mg/kg bodyweight, approximately half the intended dose of 3.75mg/kg. This was due to the large size of animals used in the study and their lower feed intake/bodyweight ratio. This is equivalent to 32ppm in feed in younger pigs rather than the usual 75ppm and highlights the need to adjust the inclusion rate to achieve the target dose.

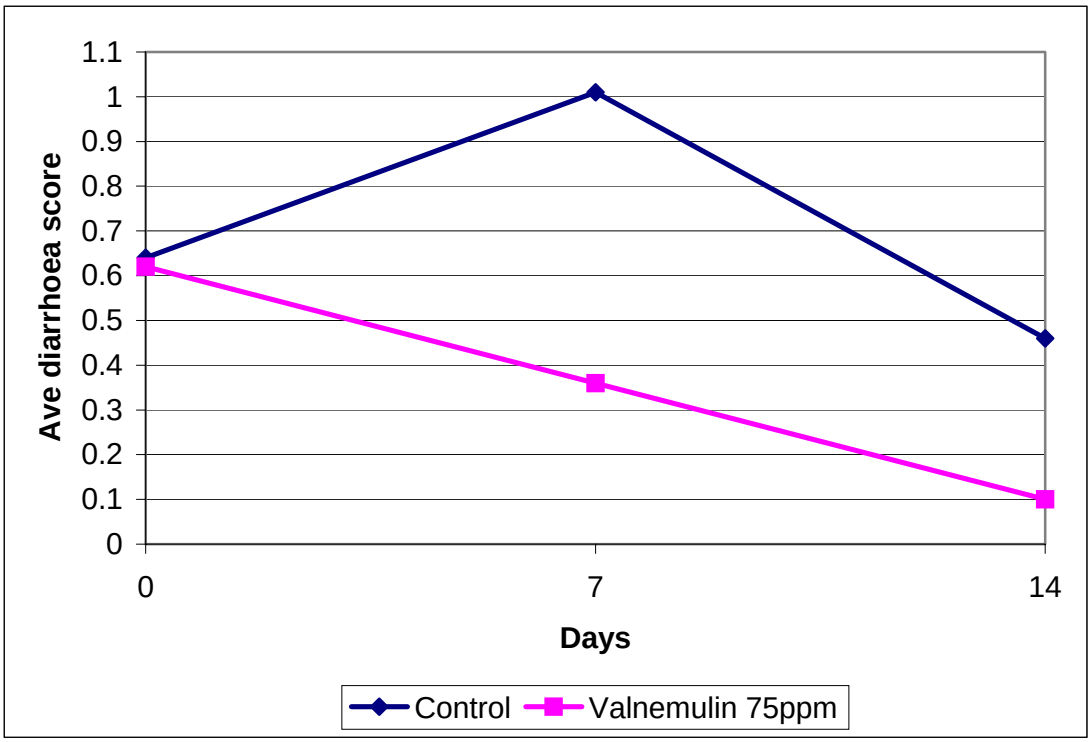
A third field study was carried out in the UK (Jones *et al*, 2004) on a small farm with a history of ileitis. To build up the numbers, the trial was carried out in two phases and the results combined. Each treatment group comprised 75 pigs, one was given valnemulin at 75ppm for 14 days and the other remained as an untreated control. The trial lasted 14 days and the pigs were weighed at the beginning and end, with the feed eaten recorded. Clinically, the pigs were examined for diarrhoea on a regular basis and samples were taken for PCR testing and confirmation of the presence of *L. intracellularis*.

Table 15 - Results of a UK field trial with valnemulin 75ppm for the treatment of ileitis (Jones *et al*, 2004)

	Untreated control	**Valnemulin 75ppm**
Average diarrhoea score day 0	0.64	0.62
Day 7	1.01	0.36
Day 14	0.46	0.10
ADG (g) day 0-14	526	626* (19%)
FCE days 0-14	2.28	1.94 (-15%)
Cumulative PCR positive samples (%)	16	3

Key: *p = <0.05

Graph 3 - Average diarrhoea score results with valnemulin 75ppm for the treatment of ileitis (Jones *et al*, 2004)



Valnemulin 75ppm in feed was effective in reducing the clinical signs of ileitis; although there was some self-cure taking place in the untreated controls. There was a reduction in the shedding of the organism and a 19% improvement in growth rate and a 15% improvement in FCE.

Conclusions - ileitis

Valnemulin has a low MIC in comparison with many other antimicrobials. In challenge studies, there appears to be a dose effect between 25-75ppm with 50ppm preventing gross lesions in a milder more typical challenge, but not completely removing histopathological lesions. All levels prevented the severe mortality seen in the more severe challenge model. A concentration of 75ppm valnemulin in a milder challenge was highly effective in treating the disease and eliminating the lesions and infection. In field studies, there was good reduction of the clinical signs of the disease, diarrhoea and bacterial shedding and exceptional improvements in growth rate and FCE.

Overall conclusions

Valnemulin at 25ppm is highly effective for the prevention of colitis caused by *B. pilosicoli* and, coupled with its claim for swine dysentery prevention and

reduction of the clinical effects of ileitis, will be particularly useful for the prevention of possibly 67% of grower diarrhoeas. Valnemulin at 75ppm is highly effective for the treatment of ileitis and, coupled with its treatment of swine dysentery and colitis, will prove highly effective in treating most grower diarrhoeas associated with valnemulin-susceptible organisms.

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