

Denagard/Tiamutin Performance

Issue 7: Ileitis *in vitro* studies

***In vitro* studies show that *L. intracellularis*, the cause of ileitis, is sensitive to Denagard®/Tiamutin®**

KEY POINTS

- Ileitis, a disease caused by the pathogen *Lawsonia intracellularis*, remains a widespread and costly problem in pigs worldwide.
- *In vitro* tests with *Lawsonia intracellularis* strains isolated in Europe and United States demonstrate their high susceptibility to tiamulin.
- Tiamulin, the active ingredient in Denagard/Tiamutin, is the most active antimicrobial inhibiting the intracellular and extracellular activity of *Lawsonia intracellularis*.
- These *in vitro* test results are consistent with the clinical response to Denagard/Tiamutin observed in the field and in many *in vivo* studies in the case of *Lawsonia*-based infections.

Introduction

Ileitis, also known as proliferative enteropathy, is a disease that affects growing and finishing pigs. It is caused by the obligate intracellular bacterium *Lawsonia intracellularis* and is a costly disease because it results in poor appetite, poor growth and sometimes scours. Ileitis can also be present in an acute form called proliferative haemorrhagic enteropathy, which usually strikes older pigs and can lead to acute intestinal haemorrhage and sudden death. Sows can have subclinical *L. intracellularis* and be carriers of the pathogen¹.

The incidence and severity of ileitis is thought to have increased worldwide, although this apparent change in pattern may represent a switch from the chronic to more acute form of the disease¹.

Until 1995, little research had been conducted or published regarding antibiotic sensitivity to *L. intracellularis*, which hindered effective treatment of ileitis in the field. Ileitis was treated with a wide range of antibiotics and recommendations for treatment were not based on *in vitro* or *in vivo* susceptibility testing².

When *in vitro* studies were conducted in the 1990s by Dr. Steven McOrist and colleagues, they showed that tiamulin, the active ingredient in Denagard/Tiamutin and a type of pleuromutilin antibiotic, was highly active against *L. intracellularis*. These study results are confirmed by new data generated with European and US strains of *Lawsonia intracellularis*.

Denagard/Tiamutin (tiamulin) concentrates inside cells where *L. intracellularis* lives, as the pathogen's name implies.



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The right choice

In vitro studies

Development of an *in vitro* cell culture system necessary for the growth of *L. intracellularis* made it possible to test antibiotic sensitivity to the pathogen. In a study, Dr. McOrist and Dr. Connie J. Gebhart, assessed the sensitivity of several antibiotics to *L. intracellularis* by determining the minimum inhibitory concentration (MIC) for each; the investigators also determined the minimum bactericidal concentration (MBC) for several selected antibiotics³. The MIC was defined as the lowest concentration that prevented multiplication of 99% of *L. intracellularis* isolates. The MBC was the lowest concentration where use of a 'pulse' antibiotic treatment stopped the growth of *L. intracellularis*.

Up to three isolates of *L. intracellularis* were used to test each antibiotic. Both intracellular and extracellular testing was conducted. Results for four of the antibiotics tested appear in Table 1 and highlight tiamulin's low MIC of 4 mcg/ml.

Table 1. MICs (mcg/ml) of various antibiotics for *L. intracellularis*, the cause of ileitis

| Antibiotic | No. of strains tested | MIC (mcg/ml) | |
|-------------------------|-----------------------|------------------------------|------------------------------|
| | | Intracellular activity assay | Extracellular activity assay |
| Tylosin tartrate | 3 | 64 | 64 |
| Lincomycin HCl | 2 | 32 | 32 |
| Spectinomycin | 1 | 32 | 32 |
| Tiamulin | 3 | 4 | 4 |

In addition to a low MIC, tiamulin also had a MBC of <2 mcg/ml, compared to 4 mcg/ml for tylosin.

Penicillin and fluoroquinolones had low MIC and MBC values in the study, but are not widely recommended for ileitis treatment in swine. In addition, antibiotics such as penicillin are used in humans, and their use in swine raises concerns about the development of antibiotic resistance in people. Denagard/Tiamutin is not used in humans.

McOrist and Gebhart note that although a few clinical reports suggested that bacitracin or aminoglycosides such as neomycin were useful in the treatment of ileitis, their MIC and other data would not support their use for *L. intracellularis*.

In 1998, McOrist and colleagues reported on additional *in vitro* testing of tiamulin, which confirmed the drug's MIC was again 4 mcg/ml when tested against *L. intracellularis*⁴.

In a new study, Dr. S. Wattanaphansak and Dr. C. Gebhart determined minimum inhibitory concentrations for several antibiotics against ten *Lawsonia intracellularis* strains from the United States (n=6) and Europe (n=4). All antimicrobials were tested in a range of different concentrations (0.25 to 128 µg/ml), which makes a titration of low and high MICs possible.

The MIC of each antimicrobial against *L. intracellularis* was identified as the lowest concentration that inhibited 99% of *L. intracellularis* proliferation as indicated by heavily infected cells. Cells were considered in the study to be heavily infected cells if the number of *L. intracellularis* proliferated inside the cell was greater than 30 bacteria per cell compared to the antimicrobial-free control.

Table 2. Intracellular and extracellular MIC ranges for *L. intracellularis* strains from the United States and Europe ⁵

| Antimicrobial agent | US <i>L. intracellularis</i> isolates (n=6) | | European <i>L. intracellularis</i> isolates (n=4) | |
|---------------------|---|----------------------------|---|----------------------------|
| | Intracellular MIC (mcg/ml) | Extracellular MIC (mcg/ml) | Intracellular MIC (mcg/ml) | Extracellular MIC (mcg/ml) |
| Chlortetracycline | 4-64 | 32-64 | 0.25-16 | 16-64 |
| Lincomycin | 16->128 | >128 | 8-64 | 32->128 |
| Tylosin | 0.25-32 | 1->128 | 0.5-2 | 2-16 |
| Tiamulin | 0.125-0.5 | 1-32 | 0.125 | 1-4 |

Intracellular and extracellular MIC assays for *L. intracellularis* were performed to mimic the real infectious situation in which *L. intracellularis* are exposed to antimicrobials before and after invasion into the pig small intestinal cells.

The results of four antimicrobials which are commonly used for the treatment and control of ileitis are summarized in Table 2.

The intracellular MICs for all antimicrobials were lower than the extracellular MICs.

Tiamulin was the most active antimicrobial inhibiting the intracellular activity of all *L. intracellularis* isolates at < 0.5 mcg/ml. The extracellular activity results indicate the highest sensitivity to tiamulin and lower MIC ranges for tiamulin in comparison to the other antimicrobials tested. An interesting observation from this study was that the intracellular and extracellular MICs for the European *L. intracellularis* strains tended to be lower than the US isolates. The authors conclude that each *L. intracellularis* strain has a different antimicrobial susceptibility pattern.

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Conclusion

In vitro studies show that Denagard/Tiamutin has low MIC values when tested against *L. intracellularis*, the cause of ileitis in swine. Not surprisingly, *in vivo* studies have also shown that Denagard/Tiamutin is effective against *L. intracellularis*. Denagard/Tiamutin works by disrupting bacterial ribosome activity and inhibiting the protein formation.

References

- 1 McOrist S, et al. Global patterns of porcine proliferative enteropathy. *The Pig Journal*. 2003;51;26-35.
- 2 McOrist S, et al. Antimicrobial susceptibility of ileal symbiont *intracellularis* isolated from pigs with proliferative enteropathy. *Journal of Clinical Microbiology*. 1995;33;5;1314-1317.
- 3 McOrist S, Gebhart, C. *In vitro* testing of antimicrobial agents for proliferative enteropathy (ileitis). *Swine Health and Production*, July and August, 1995, page 146-149.
- 4 McOrist S, et al. *In vitro* and in-life studies of efficacy of valnemulin for proliferative enteropathy (ileitis). *15th International Pig Veterinary Society Congress*, Birmingham, England 1998.
- 5 S.Wattanaphansak, D.Rau, R.Singer, C.Gebhart. *In vitro* activity of six antimicrobial agents against ten *Lawsonia intracellularis* isolates from the United States and Europe. 3rd Asian Pig Veterinary Society Congress, Wuhan, China 2007.