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IN VITRO STUDIES SHOW SENSITIVITY OF *L. INTRACELLULARIS* TO VALNEMULIN

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INTRODUCTION Despite modern swine production practices, ileitis remains a significant concern for pork producers around the globe, resulting in huge production and economic losses. The disease, more specifically known as porcine proliferative enteropathy, tends to affect growers and finishers and results in problems ranging from bloody scours and poor growth to slaughter condemnation and sudden death. Effective methods of controlling and treating ileitis are urgently needed.

Ileitis is caused by the bacterial pathogen *Lawsonia intracellularis*. Even though the disease has been a problem in the swine industry for over 20 years, the pathogen has demonstrated no clear pattern of antibiotic sensitivity. Consequently, investigator Steven McOrist and colleagues conducted *in vitro* studies to determine which antibiotics are and are not effective against *L. intracellularis*.

McOrist's *in vitro* studies indicate that some drugs used to treat ileitis in the field are not likely to be highly effective but that a newer class of drugs — pleuromutilins — may offer effective control. Pleuromutilins are drugs such as tiamulin as well as valnemulin, the main ingredient in Econor[®]. Econor is approved in several countries for preventing and treating swine dysentery and recently was approved in Europe for preventing colitis and treating ileitis. Valnemulin and tiamulin are not antimicrobials used in humans, which helps reduce concerns about the development of antibiotic resistance in people.

MICs for Various Antibiotics

In an initial *in vitro* study, McOrist studied the effect of 18 antimicrobial agents.¹

The minimum inhibitory concentration (MIC) of each agent was determined for up to three *L. intracellularis* isolates. The MIC is the lowest concentration of an antimicrobial needed to inhibit the growth of a microorganism.

Both an intracellular and extracellular activity assay was obtained. The *L. intracellularis* isolates were obtained from lesions of proliferative enteropathy and were cultured in rat enterocyte cultures utilizing a process known to be reliable. The investigators also determined minimum bactericidal concentrations (MBC) for selected antimicrobials using one of the *L. intracellularis* isolates. The MBC is the lowest concentration where use of a 'pulse' treatment of antimicrobial agent stops the growth of *L. intracellularis*.

Valnemulin was not one of the antimicrobials included in this study because it was too new at

KEY POINTS

- > *In vitro* testing of the pleuromutilin tiamulin revealed a MIC of only 4 mcg/mL (see Table 1) and an MBC of < 2 mcg/mL, indicating sensitivity of *L. intracellularis* to the antimicrobial.
- > The MIC value for the newer pleuromutilin valnemulin was only < 2 mcg/mL.
- > Valnemulin's low MIC value, coupled with its *in vivo* efficacy demonstrated in other studies, indicates that the antibiotic has an active bacteriostatic and possibly bacteriocidal effect against *L. intracellularis*.

the time and not approved for use in the United States, where the study was conducted.

Results

> The pleuromutilin tiamulin had a MIC of only 4 mcg/mL (see Table 1) and an MBC of < 2 mcg/mL.

> There was variation in the results among macrolides (Table 1), similar to those noted in *in vitro* MIC studies for other intracellular bacteria.

> The aminoglycosides tested, such as neomycin, as well as the peptides (bacitracin, avoparcin) and cephalosporins (ceftiofur) had MIC/MBCs greater than a likely dose rate for use in pigs, indicating poor *L. intracellularis* sensitivity to these compounds.

> The most active compounds tested included penicillin and fluoroquinolones, but these are not widely recommended for treating ileitis in swine.

Some of the drugs, such as penicillin, are used in humans, and their use in swine raises concerns about the development of antibiotic resistance in people.

Researchers' observations: Tiamulin and some of the macrolides had low to moderate MIC/MBC values. They act by inhibiting the protein synthesis of bacteria. *L. intracellularis* is not susceptible to aminoglycosides.

MICs for Valnemulin

In additional *in vitro* studies,² McOrist and associates studied the antimicrobial effect of valnemulin on *L. intracellularis*. The bacterium was co-cultured in intestinal rat epithelial cell lines and the results were compared to those for tiamulin and chlortetracycline. To provide a control, they included co-cultures of *L. intracellularis* without antibiotic in the study.

Results

> The MIC for valnemulin was < 2 mcg/mL. That is, a concentration of only 2 mcg/mL or more of valnemulin in the media of a co-culture of *L. intracellularis* inhibited the organism's growth under standard conditions compared to controls.

> The MIC for tiamulin was 4 mcg/mL.

Discussion

In an expert report,³ McOrist points out that pig enterocytes for MIC studies have not been available or developed, but rat enterocyte co-cultures offer a "clearly identical metabolic comparison." The lesions of ileitis and the location and metabolism of *L. intracellularis* in rat enterocytes is similar to the disease in pigs, indicating the non-specific nature of *Lawsonia sp.* metabolism in different host enterocytes, McOrist says.

In addition, even though only a small number of *L. intracellularis* isolates from Europe have been MIC-tested, detailed examination indicates they

Table 1

MICs and MBCs of antimicrobial agents for *L. intracellularis*

| Antibiotic | No. Strains Tested | Intracellular Activity Assay MIC (mcg/mL) | Extracellular Activity Assay MIC (mcg/mL) | MBC (mcg/mL) |
|-------------------------|--------------------|---|---|--------------|
| Peptides | | | | |
| Bacitracin-zinc | 1 | > 32 | > 32 | * |
| Macrolides | | | | |
| Tylosin tartrate | 3 | 64 | 64 | 4 |
| Erythromycin | 2 | 0.1 | 0.5 | * |
| Lincosamide | | | | |
| Lincomycin HCl | 2 | 32 | 32 | * |
| Aminoglycosides | | | | |
| Neomycin sulfate | 3 | > 128 | 128 | * |
| Gentamicin sulfate | 2 | > 128 | 128 | > 50 |
| Penicillin | | | | |
| (Penicillin G procaine) | 2 | 1 | 2 | 4 |
| Fluoroquinolones | | | | |
| Difloxacin HCl | 2 | 0.1 | 0.5 | < 2 |
| Enrofloxacin | 2 | 8 | 8 | 4 |
| Pleuromutilin | | | | |
| Tiamulin | 3 | 4 | 4 | <2 |

* Not tested.

The MIC for valnemulin was only < 2 mcg/

are all basically the same single "strain." This indicates, writes McOrist, that MIC or other similarly derived data sets are likely to be accurate between isolates.

SUMMARY

L. intracellularis has been shown to be relatively sensitive to certain compounds, including pleuromutilins such as valnemulin, which act against the bacterial ribosomes, McOrist concludes.

Results of *in vitro* and *in vivo* studies indicate that valnemulin and tiamulin can penetrate pig intestinal epithelial cells, enter the intracytoplasmic *Lawsonia* bacteria and cause bacterial death by disruption of the bacterial ribosome activity.

Valnemulin's low MIC value, coupled with its *in vivo* efficacy demonstrated in other studies, indicates that the antibiotic has an active bacteriostatic and possibly bacteriocidal effect against *L. intracellularis*, the pathogen responsible for ileitis in swine, he says.

For more information on these studies, please contact your local Novartis Animal Health representative or Dr. Ulrich Klein at Ulrich.klein@ah.novartis.com

REFERENCES

¹ McOrist S, Gebhart C. *In vitro* testing of antimicrobial agents for proliferative enteropathy (ileitis). *Swine Health and Production*. 1995;3:147-149.

² McOrist S, et al. *In vitro* and in-life studies of efficacy of valnemulin for proliferative enteropathy (ileitis). *The 15th International Pig Veterinary Society Congress*. 1998.

³ McOrist S. Evaluation of the efficacy of valnemulin against the causative agent of proliferative enteropathy in pigs, *Lawsonia intracellularis*. *Preclinical Documentation and Expert Report and Comment*. 2003.

mL and the MIC for tiamulin only 4 mcg/mL.



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