Zuprevo® –
A new paradigm in the treatment and prevention of BRD

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Bovine Health Management
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Pharmaceutical R&D at MSD Animal Health
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Preface

The new MSD Animal Health is delighted to welcome you to our symposium at the 6th European Congress of Bovine Health Management in this beautiful setting along the river Meuse in the heart of Liège. On this occasion, we are pleased to introduce a new tool called “Zuprevo®” which will help veterinarians and cattle producers around the world to be in control of bovine respiratory disease (BRD).

Scientists of MSD Animal Health (formerly Intervet/Schering-Plough Animal Health) in cooperation with their partners in academia and contract research organizations have played a crucial role in the discovery and development of this novel tool.

Although bovine veterinarians have many choices available for treatment and prevention, BRD remains a very frequent and economically important disease in cattle. BRD is a multi-factorial disease, and cattle are prone to rapidly develop severe lung pathology. Factors triggering BRD outbreaks include the anatomical predisposition of the bovine respiratory system, common stress inducing cattle management practices such as weaning, commingling, and transportation, as well as suboptimal housing and climatic conditions. Furthermore, the bacterial respiratory pathogens are ubiquitous and appear unlikely to ever be eradicated.

Findings from advanced diagnostic procedures, specifically the monitoring of intra-ruminal temperature, suggest that visual detection of BRD very often occurs when the disease is already well advanced. The initiation of curative treatment is thus often late while at the same time BRD pathogens may have been rapidly spreading amongst the untreated animals in the herd. Therefore, for both treatment and prevention, it is important for the antimicrobial to be present at the site of infection, i.e. lung tissue and bronchial fluid, both rapidly and for an extended period of time.

Zuprevo® is a novel tri-basic 16-membered-ring macrolide with unique pharmacokinetic and pharmacodynamic characteristics, available in a highly convenient cattle specific formulation, addressing both the need for speed in the treatment and for duration in the prevention of BRD.
In collaboration with MSD Animal Health, Professor Stephen Douthwaite, a molecular microbiologist from the University of Southern Denmark, Odense, intensively studied the mode of action of the active ingredient of Zuprevo®, tildipirosin. In his presentation, he will reveal well illustrated findings based on footprinting experiments and molecular modeling as a basis of the improved antibacterial activity of tildipirosin and its unique pharmacodynamic properties.

The key features of the Zuprevo® product profile will be presented by Dr. Rainer Röpke. He managed the research and development activities for Zuprevo® as global project manager at MSD Animal Health. Besides highlighting the benefits of a convenient formulation, a high margin of safety and a comparatively short withdrawal period, Dr. Röpke will discuss the unique pharmacokinetic profile of tildipirosin in lung tissue and bronchial fluid, which contributes to the excellent clinical efficacy demonstrated in field trials across the EU.

Dr. Markus Rose, Senior Development Manager at MSD Animal Health, will describe the findings from a Mannheimia haemolytica challenge study demonstrating the principle of Zuprevo®’s preventive efficacy in BRD, in comparison to Draxxin®. The superior clinical and antibacterial efficacy of Zuprevo® observed in this study confirms its outstanding pharmacodynamic and pharmacokinetic properties.

Professor Kerstin E. Müller from the Faculty of Veterinary Medicine at the Free University of Berlin will present a field trial demonstrating the excellent therapeutic efficacy of Zuprevo® against BRD in young calves, in comparison to Draxxin®. This study confirms the fast onset of action and provides further compelling clinical evidence for the excellent clinical efficacy of Zuprevo® in a real world setting.

MSD Animal Health is committed to be a leader in innovative research and to be a partner to veterinary practitioners and livestock producers globally. We are therefore very pleased to be your host at this symposium and to share with you this information on Zuprevo®, a new standard in the treatment and prevention of BRD.

We wish you a very enjoyable evening.

Dr. Peter Schmid
Head of Drug Discovery
MSD Animal Health
**Prof. Stephen Douthwaite**

Professor Stephen Douthwaite studied biochemistry and microbiology at the University of Wales in Cardiff (1976). Soon afterwards, he joined the Max Planck Institute in West Berlin where he worked on ribosomal protein synthesis and his research interests developed to include antibiotic inhibitors of this process. He obtained his PhD from Aarhus University in Denmark (1982), and spent three years as a postdoc at the University of California in Santa Cruz. He is presently Professor of Molecular Microbiology at the University of Southern Denmark, Odense, where he has been employed since 1987.

**Dr. Rainer Röpke**

Dr. Rainer Röpke studied Agriculture at the Technical University of Munich and the University of Göttingen, Germany, where he earned his diploma in 1989. In 1992 he obtained his PhD from the Institute for Animal Physiology and Research Centre for Milk and Food at the Technical University of Munich. Starting in the animal health industry in 1992 he worked as Global Regulatory Affairs Manager responsible for Feed Additives at Hoechst Roussel Vet in Wiesbaden, Germany, before relocating in 2000 to join their US organization (then Intervet Inc.) in Delaware, USA, as Director Pharmaceutical Development. Since 2003 he has been Global Project Manager at Intervet Innovation GmbH in Schwabenheim, Germany (now member of the MSD Animal Health Group), managing all activities in relation to the global development of new pharmaceutical products.
Dr. Markus Rose

Dr. Markus Rose graduated from the veterinary faculty of the Justus-Liebig-University (JLU), Gießen, Germany, in 1987, and worked in private veterinary practice till 1989. Between 1990 and 1993 he was research associate at the Veterinary Institute of the Karl-August-University, Göttingen, Germany, specializing in microbiology. He received his doctorate in 1991. During his employment with Hoechst Roussel Vet GmbH, Wiesbaden, Germany, beginning in 1994, he managed clinical study programs, and relocated to the US in 1998, finally serving as senior project leader. Back in Germany in 2002, at Intervet Innovation GmbH (now member of the MSD Animal Health Group), he continued with project development, and was responsible for a group providing clinical study support. Since 2004 he has been senior development manager with focus on microbiological, food safety and clinical aspects of global pharma projects.

Prof. Dr. Kerstin E. Müller

Professor Kerstin E. Müller earned her degree from the Veterinary University Hannover in 1984. She obtained her doctoral degree in 1986 and was recognized as German cattle specialist in 1989 when she was member of the Cattle Clinic in Hannover. In 1995 she obtained the PhD from Utrecht University, NL, where she was Senior Lecturer for Internal Medicine of Ruminants and Horses from 1991 to 1998 and subsequently member of the Department of Farm Animal Health with certification as a Dutch Cattle Specialist. Since 2003 she has been Professor and Managing Director at the Clinic for Ruminants and Swine at the Faculty of Veterinary Medicine, Freie Universität Berlin. She is chair of the Education and Residency Committee of the ECBHM and of the German Buiatrics Association and board member of the WBA.
1.
Tildipirosin – Mode of Action of a Novel Macrolide

Stephen Douthwaite, Jacob Poehlsgaard and Ralf Warrass
Tildipirosin – Mode of Action of a Novel Macrolide

Stephen Douthwaite¹, Jacob Poehlsgaard¹ and Ralf Warrass²

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² Drug Discovery, Intervet Innovation GmbH, a member of the MSD Animal Health Group, Zur Propstei, D-55270 Schwabenheim, Germany.

Abstract

MSD Animal Health has recently been granted market-authorization in the European Union for Zuprevo® in the treatment and prevention of respiratory disease in cattle. The active ingredient tildipirosin is the first tri-basic 16-membered-ring macrolide on the market. The lipophilic – hydrophilic flexibility of the three basic centres of tildipirosin are made responsible for its high solubility, rapid tissue distribution, extensive accumulation in lung tissue and excellent antibacterial activity against bacteria associated with bovine respiratory disease. Tildipirosin’s antimicrobial activity is achieved by effective penetration of the bacterial cell wall and binding to the molecular target, the bacterial ribosome. Using chemical footprinting experiments and molecular modelling we compared tildipirosin’s ribosomal attachment to that of other macrolides (tylosin, tilmicosin, tulathromycin) and found common interaction sites but also clear differences and unique binding modes. In an in vitro transcription/translation assay, tildipirosin demonstrated highly efficient inhibition of ribosomal peptide synthesis with an IC₅₀ of 0.23 (± 0.01) µM reflecting the improved structural fit of this molecule. This is significantly better than e.g. tilmicosin with an IC₅₀ of 0.36 (± 0.02) µM.

Macrolides comprise a large family of naturally-occurring compounds that exhibit a range of therapeutic properties, the best-characterized and presently most important of which is their antimicrobial activity. From a chemical standpoint, macrolides are made up of a macrolactone ring, consisting of 12 to 16 atoms, which is substituted with up to three sugar moieties. The most successful macrolides in hospitals and in the field are chemical derivatives of their natural progenitors with improved stability, efficacy, safety and convenience. The latest of these is tildipirosin, a new chemical entity (NCE) for the management of bovine and swine respiratory diseases. Tildipirosin has been shown to be particularly efficacious against respiratory tract infections caused by the Gram-negative bacterial pathogens Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae and Haemophilus parasuis.

Tildipirosin is presently the only tri-basic 16-membered-ring macrolide on the market. The chemical structure of tildipirosin has retained the tylosinolide macrolactone core from the natural product tylosin, but differs in several other aspects, the most obvious of which are two piperidine substituents (Fig. 1). One piperidine has been added to the 20-position of the tylosinolide ring, while the other replaces the 23-mycinosyl sugar. In addition, the mycamethylo-mycarose disaccharide at the C5-atom has been shortened to remove the mycarose sugar. The resultant structure contains three basic centres, with pKa values of 9.8, 8.8 and 8.1. The structure of tildipirosin exhibits both lipophilic and hydrophilic characteristics, which vary depending on the pH of the environment and interaction with membranes and proteins. This imparts several beneficial properties including higher solubility, improved penetration into tissues and accumulation at the site of infection (lung tissue). In addition, the effective penetration of tildipirosin through the outer-membrane of Gram-negative bacteria is one of the properties that make the drug particularly effective against pathogens such as M. haemolytica and P. multocida.

Figure 1. Chemical structure of the tri-basic 16-membered-ring macrolide tildipirosin.
While improved cell penetration is an important characteristic, a successful antimicrobial compound also needs to be an effective inhibitor of bacterial metabolism. Similar to other macrolides, tildipirosin inhibits the process of translation – the synthesis of new peptides and proteins by the bacterial ribosome (see Fig. 2).

Using an in vitro translation system with ribosomes from the Gram-negative bacterium *Escherichia coli*, we showed that 50% of protein synthesis was inhibited by tildipirosin at 0.23 µM (IC$_{50}$ = 0.23 ± 0.01 µM) compared to another 16-membered-ring macrolide tilmicosin, which was shown to have an IC$_{50}$ value of 0.36 ± 0.02 µM (see Table 1).

<table>
<thead>
<tr>
<th>16-membered macrolides</th>
<th>IC$_{50}$* (mean ± standard deviation)</th>
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<tbody>
<tr>
<td>Tildipirosin</td>
<td>0.23±0.01 µM</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>0.36±0.02 µM</td>
</tr>
</tbody>
</table>

* Concentration at which 50% of protein synthesis is inhibited

Table 1. Inhibition of ribosomal peptide synthesis as determined in a coupled transcription/translation system with ribosome from *E. coli*.

Establishing that tildipirosin was an effective inhibitor of protein synthesis led to the questions of what molecular interactions of tildipirosin occur at its site of inhibition on the ribosome, and whether these interactions differ from those of the other macrolides. We addressed these issues in two steps: First, the sites of macrolide drug contact were mapped within the ribosome structure using chemical footprinting, and these data were then used computationally in molecular dynamic simulations to visualize how the drugs occupy and are oriented within their binding site on the ribosome. In the case of tyllosin, a high resolution crystal structure of the drug bound to its ribosomal site is available (PDB: 1K9M). We used this information to verify and to validate our method of molecular dynamic simulation. Our independently derived simulation for tyllosin binding to the ribosome is compared with that of the crystal structure in Fig. 3, showing remarkably consistent data sets.

The footprinting data showed that the macrolide drugs tildipirosin, tilmicosin and tyllosin bind in the same region of *Pasteurella* and *E. coli* ribosomes, the latter being the standard model for this type of study. All macrolides interact with the ribosomal RNA and at nucleotides A2058 and A2059 that are located within the tunnel of the large ribosomal subunit. This observation was expected from previous studies on the mode-of-action of other macrolide compounds (e.g. Poulsen, *et al.* 2000. *J Mol Biol*, 304: 471–481); however, at neighbouring nucleotides there were distinguishing differences between the contacts made by tildipirosin, compared to the other macrolides studied.
The two piperidine rings in tildipirosin provide the potential for unique interactions (Fig. 4). Importantly, the 20-piperidine is oriented into the lumen of the ribosome tunnel at a location that is not occupied by a substituent found in any other macrolide. From this position, the 20-piperidine could potentially block the growth of the newly synthesized peptide chain as it passes down the tunnel. This model is consistent with the IC₅₀ data, which show a higher potency of tildipirosin (compared to tilmicosin) to inhibit protein synthesis on the bacterial ribosome reflecting the improved structural fit of this new tri-basic 16-membered-ring macrolide to its molecular target.

Notes
2. Zuprevo®: Key features of a unique product

Rainer Röpke, Monika Menge, Markus Rose, Andreas Ehinger, Cornelia Bohland, Cornelia Wilhelm, Eva Zschiesche, Susanne Kilp, Wibke Metz and Mark Allan
Zuprevo®: Key features of a unique product

Rainer Röpke, Monika Menge, Markus Rose, Andreas Ehinger, Cornelia Bohland, Cornelia Wilhelm, Eva Zschiesche, Susanne Kilp, Wibke Metz and Mark Allan

Intervet Innovation GmbH, a member of the MSD Animal Health Group

Abstract

MSD Animal Health has recently been granted marketing authorization in the European Union (EU) for Zuprevo® for the treatment and prevention of respiratory disease in cattle. Its active ingredient, tildipirosin, is the first tri-basic 16-membered-ring macrolide. The cattle-specific formulation is a ready-to-use, sterile aqueous solution containing 180 mg of tildipirosin/mL for use as a single subcutaneous injection (4 mg/kg BW) not to exceed 10 mL per injection site, equivalent to 450 kg BW. The unique pharmacokinetics of Zuprevo® are characterized by rapid absorption from the injection site and long-lasting high concentrations in the lung and bronchial fluid. Mean maximum plasma concentrations (Cmax) are reached as early as 23 min (Tmax) post injection and its terminal plasma elimination half life (T½) is about 9 days. With about 50 L/kg, the volume of distribution after IV administration is exceptionally large in comparison to other long-acting macrolides, while its clearance of 144 mL × h/kg is comparatively low. The MIC90s of tildipirosin are 1 µg/mL for M. haemolytica and P. multocida and 4 µg/mL for H. somni. Tildipirosin concentrations in lung tissue exceed the H. somni MIC90 for about 16 days and the MIC90s for M. haemolytica and P. multocida for at least 28 days. Zuprevo®'s high margin of safety has been demonstrated in studies exceeding the label dose of 4 mg tildipirosin/kg BW up to 10 fold. Furthermore, safety for the consumer is demonstrated by the high acceptable daily intake (ADI) of tildipirosin (6000 µg/person/day) derived from a comprehensive package of toxicological and microbiological safety studies; the ADI is about 10 times higher than that of tulathromycin (660 µg/person/day, Draxxin®: EPAR) and gamithromycin (600 µg/person/day, Zactran®: EPAR). This is reflected in a shorter withdrawal period of 47 days for Zuprevo® in comparison to other macrolides (49 days, Draxxin®: EPAR; 64 days, Zactran®: EPAR).
What differentiates Zuprevo® further from other macrolides is its unique pharmacokinetic profile. Pharmacokinetics of Zuprevo® have been investigated extensively following SC administration at different doses, in different matrices and following IV versus SC administration. Rapid absorption, extensive distribution and long elimination half-lives are the key pharmacokinetic features of Zuprevo®. Mean maximum plasma concentrations (C_{\text{max}}) were reached as early as 23 min (T_{\text{max}}) after SC administration of 4 mg tildipirosin/kg BW. In comparison to published plasma-concentration-versus-time profiles of Draxxin® (Nowakowski et al., 2004) and Zactran® (Huang et al., 2010), tildipirosin concentrations in plasma are higher and more persistent as shown in Figures 1–3. This is reflected in Zuprevo®'s terminal plasma elimination half life (T_{1/2}) of about 9 days which is substantially longer than those of Draxxin® (about 4 days, Nowakowski et al., 2004), Zactran® (>2 days, Huang et al., 2010) and Micotil®300 (about 1 day, Lombardi et al., 2010).

Macrolides are known to accumulate in lung tissue with multi-fold higher concentrations than in plasma. With about 50 L/kg, the volume of distribution of Zuprevo® after IV administration is exceptionally large in comparison to those of Draxxin® (about 11 L/kg, Nowakowski et al., 2004), Zactran® (about 25 L/kg, Huang et al., 2010) and Micotil®300 (15.3 L/kg, Lombardi et al., 2010). At the same time, the clearance of Zuprevo® (144 mL x h/kg) is lower than those of Draxxin® (180 mL x h/kg, Nowakowski et al., 2004), Zactran® (712 mL x h/kg, Huang et al., 2010) and Micotil®300 (686 mL x h/kg, Lombardi et al., 2011). These observations are confirmed by prolonged high lung-tissue concentrations of Zuprevo® in comparison to Draxxin® and Zactran® as shown in Figures 4–6.

The MIC\textsubscript{90} of tildipirosin were determined against the pathogens most commonly involved in the etiology of BRD. They were 1 µg/mL for *M. haemolytica* and *P. multocida* and 4 µg/mL for *H. somni*. MIC\textsubscript{90} values were determined under standard CLSI testing conditions in epidemiologically unrelated bovine isolates (wild-type) collected in different European countries. Similar to other macrolides such as tulathromycin, gamithromycin and tilmicosin, tildipirosin plasma concentrations were below MIC\textsubscript{90} (Nowakowski et al., 2004; Godinho, 2008; Huang et al., 2010; Modric et al., 1998; Shryock et al., 1996). Therefore, it may be concluded that the typical pharmacodynamic analyses appropriate for beta-lactam antibiotics, quinolones and aminoglycosides do not apply in the context of the activity of the newer macrolides (Nightingale, 1997). Other potential pharmacodynamic properties of this drug group include stimulation of cytokine pathways and enhancement of programmed cell death (apoptosis) (Lees et al., 2006). Such actions – in addition to the direct bactericidal effects – may exert additional or synergistic beneficial effects in infectious lung diseases.

Tildipirosin concentrations in lung tissue exceed the MIC\textsubscript{90} of *H. somni* for about 16 days and the MIC\textsubscript{90} of *M. haemolytica* and *P. multocida* for at least 28 days which is much longer compared to Draxxin® and Zactran® as shown in Table 1. In field trials in the EU, Zuprevo®’s clinical efficacy has been demonstrated for periods of 21 days. Predictions of efficacy based on lung homogenate concentrations are subject to scientific discussions (Toutain, 2009). All 3 respiratory pathogens targeted by tildipirosin attach to bronchial epithelial cells and remain extracellular. Therefore, bronchial fluid obtained from live, non-anesthetized cattle at serial time points was analysed to demonstrate bacterial exposure to drug concentrations at the site of infection. A method was applied on the basis of procedures described by Halstead et al. (1991) which allows the safe collection of bronchial fluid samples void of blood and unaffected by dilution factors. Bronchial fluid samples were taken at serial time points post-dose in the same non-anesthetized animals. Mean bronchial fluid tildipirosin concentrations exceeded the MIC\textsubscript{90} of *M. haemolytica* and *P. multocida* for 21 days, and approximated the MIC\textsubscript{90} of *H. somni* for about 3 days. The concentration-versus-time profiles of mean tildipirosin concentrations in plasma, bronchial fluid and lung tissue are summarized in Figure 7.

While protein binding of tildipirosin in bovine bronchial fluid is only 30%, it is acknowledged that drug concentrations measured in bronchial fluid using the method described above represents a conglomerate of intracellular and extracellular drug contents. As macrolides are known to accumulate within phagocytic cells (Gladue et al., 1989; Scorneaux & Shryock, 1998), tildipirosin concentrations determined in bronchial fluid also reflect that portion of respiratory drug accumulation. Bronchoalveolar lavage (BAL) has been described by others as a

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method to determine extracellular drug concentrations in the pulmonary epithelial-lining fluid (PELF) and intracellular drug concentrations in the BAL cells (Cox et al., 2010; Giguère et al., 2011). However, the analytical procedures remain a point of discussion (Zeitlinger et al., 2005; Kiem & Schentag, 2008), thus further research is required to accurately describe the PK/PD relationships of macrolides used in veterinary medicine including those of tildipirosin.

In conclusion, Zuprevo® is a novel 16-membered-ring, tri-basic macrolide with unique pharamacokinetics, a high margin of safety, and a comparatively short withdrawal period, offered in a convenient formulation.

References:
Tables and Figures

**Figure 1.** Plasma concentration-time curve of tildipirosin (Zuprevo®) after SC administration of the product label dose.

**Figure 2.** Plasma concentration-time curve of tulathromycin (Draxxin®) after SC administration of the product label dose.

**Figure 3.** Plasma concentration-time curve of gamithromycin (Zactran®) after SC administration of the product label dose.

**Figure 4.** Lung tissue concentration-time curve of tildipirosin (Zuprevo®) after SC administration of the product label dose.

**Figure 5.** Lung tissue concentration-time curve of tulathromycin (Draxxin®) after SC administration of the product label dose.

**Figure 6.** Lung tissue concentration-time curve of gamithromycin (Zactran®) after SC administration of the product label dose.

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**Draxxin®**


**Zactran®**


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**Consolidated plasma concentrations**

**Consolidated lung concentrations**
**Figure 7.** Consolidated concentration-time curves for tildipirosin (Zuprevo®) in lung tissue, bronchial fluid (*in vivo*) and plasma after SC administration of the product label dose.

<table>
<thead>
<tr>
<th>Days of drug concentrations in lung tissue above MIC₉₀</th>
<th>M. haemolytica (MIC₉₀)</th>
<th>P. multocida (MIC₉₀)</th>
<th>H. somni (MIC₉₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ZUPREVO®</strong></td>
<td>&gt;28 (1 µg/mL)</td>
<td>&gt;28 (1 µg/mL)</td>
<td>~16 (4 µg/mL)</td>
</tr>
<tr>
<td><strong>DRAXXIN®</strong></td>
<td>~10⁻⁶ (2 µg/mL³)</td>
<td>&gt;15⁻⁴⁻¹ (1 µg/mL³)</td>
<td>~0.5⁻¹ (4 µg/mL³)</td>
</tr>
<tr>
<td><strong>ZACTRAN®</strong></td>
<td>~15⁻⁸ (0.5 µg/mL⁻¹)</td>
<td>~12⁻¹ (1 µg/mL⁻¹)</td>
<td>~12⁻¹ (1 µg/mL⁻¹)</td>
</tr>
</tbody>
</table>

*Godinho (2008) Veterinary Microbiology, 129, 426–432
*calculated using WinNonlin® software with data derived from Nowakowski et al (2004) Veterinary Therapeutics, 5, 60–74

**Table 1.** Consolidated times (days) above MIC₉₀ of *M. haemolytica*, *P. multocida* and *H. somni* for tildipirosin (Zuprevo®), tulathromycin (Draxxin®) and gamithromycin (Zactran®) lung tissue concentrations after SC administration of the product label dose.
3. Preventive efficacy of Zuprevo® in a Mannheimia haemolytica challenge study of bovine respiratory disease

Markus Rose, Wibke Metz, Joachim Ullrich, Eva Zschiesche, Silke Sczesny, Mark Allan and Rainer Röpke
Preventive efficacy of Zuprevo®
in a *Mannheimia haemolytica* challenge study of bovine respiratory disease

Markus Rose, Wibke Metz, Joachim Ullrich, Eva Zschiesche, Silke Sczesny, Mark Allan and Rainer Röpke

*Intervet Innovation GmbH, a member of the MSD Animal Health Group*

Abstract

Preventive antimicrobial treatment is an important element in herd health programs aimed at minimizing the incidence and costs associated with Bovine Respiratory Disease (BRD). MSD Animal Health has recently been granted marketing authorization in the European Union for Zuprevo® for the treatment and prevention of respiratory disease in cattle. Its active ingredient, tildipirosin, the first tri-basic 16-membered-ring macrolide is characterized by rapid absorption from the subcutaneous injection site and long-lasting high lung and bronchial fluid concentrations. These pharmacokinetic properties make Zuprevo® an optimal choice for the prevention of BRD as demonstrated in a *Mannheimia haemolytica* challenge study. In this study, tildipirosin (4 mg/kg, Zuprevo®) was shown to prevent mortality, and was superior in terms of clinical symptoms and bacterial counts in lung tissue compared to saline and tulathromycin (2.5 mg/kg, Draxxin®).

The role of preventive treatment in bovine respiratory disease (BRD) management

BRD remains very important to the modern cattle industry given its high incidence in herds leading to substantial economic losses through the reduced performance of the affected cattle in addition to treatment costs and mortality (Babcock et al. 2010; Schneider et al. 2009). BRD outbreaks most frequently occur following transportation and commingling stress in young, newly weaned calves, often originating from many different farms and with unknown health history or immune status (Step et al. 2008; Taylor et al. 2010). The incidence of BRD in these calves peaks within the first few weeks after commingling (Edwards 2010), but BRD infection rates can remain high until approximately 80 days on feed (Snowder et al. 2006). Since commingling of calves is a common management practice, total eradication of BRD pathogens is unlikely (Snowder et al. 2006).

BRD is a multi-factorial disease caused by both infectious and non-infectious factors (Lekeux 1995; Stoeber 2002; Edwards 2010; Taylor et al. 2010):

- viral pathogens (BRSV, PI-3, BHV-1, BVD/MD)
- bacterial pathogens (*M. haemolytica*, *P. multocida*, *H. somni*, *M. bovis*)
- exo- and endogenic factors (e.g. management, physiology, environment)

Viral pathogens frequently play an initial role, predisposing cattle to secondary bacterial pathogens. However, *M. haemolytica* is also known to be a primary pathogen and has traditionally been the most commonly isolated bacterial pathogen (Taylor et al. 2010). The bacterial involvement usually turns the disease into a fibrinous or suppurrative bronchopneumonia with clinical signs of pronounced dyspnoea and fever, coughing, nasal discharge and inappetence, associated with moderate to severe depression (Stoeber 2002). However at slaughter, lung lesions may occur in the majority of untreated cattle indicating also the importance of subclinically infected animals and challenges with the visual detection of BRD (Edwards 2010).

Prevention is an important element in herd health programs aimed at minimizing the incidence and costs associated with BRD morbidity and mortality (Edwards 2010). In the EU, it is a regulatory requirement that the presence
of the disease should be confirmed in the herd before preventive treatment is initiated. E.g. a percentage of the cattle in the herd already present clinical signs of BRD whereas the others show no overt disease but may already be subclinically infected. In this regard, in a recent investigation measuring body temperature by reticulo-rumen temperature boluses, fever episodes began 4 to 177 hours before treatment upon clinical signs of BRD (Timsit et al. 2010). Therefore, to prevent irreversible lung damage and severe economic losses, early antimicrobial treatment providing rapid onset of action and a long duration of activity is crucial to cover the critical early fattening period following transportation and commingling, and to avoid further contracting of the disease in the herd.

Tildipirosin, the active ingredient of Zuprevo®, is a novel 16-membered-ring, tri-basic macrolide licensed for both the treatment and prevention of BRD. It is characterized by rapid absorption from the subcutaneous injection site and long-lasting high concentrations at the site of infection (EMA 2011). These pharmacokinetic features make Zuprevo® an optimal choice for the prevention of BRD where it is crucial that antibiotic therapy acts rapidly and provides cover over a sustained period of time to avoid further spread of the disease in the herd. The principle of preventive efficacy of Zuprevo® was demonstrated in a Mannheimia haemolytica challenge model of BRD.

Mannheimia haemolytica challenge study

Materials and methods: In dose-finding experiments, 18 healthy male Holstein black pied calves were allocated to three groups of six animals each. On Day 0, animals received the following single subcutaneous (SC) injection:

- **Group 1**: Zuprevo®, 4 mg tildipirosin/kg BW
- **Group 2**: Draxxin®, 2.5 mg tulathromycin/kg BW
- **Group 3**: Saline, 2 mL/100 kg BW.

Based on a well-established study model (Shoo, 1989), five days after treatment (Day 5) all animals were challenged by intratracheal inoculation of approximately $1 \times 10^9$ colony forming units (CFU) of *M. haemolytica* 7/2 Serotype A 1 (tildipirosin MIC of 0.25 μg/mL) (Figure 1). This pathogenic strain provided by the Moredun Research Institute, Scotland, originated from pneumonic pasteurellosis and was successfully used in other challenge studies. All animals were handled and treated in accordance with German animal welfare regulations.

Clinical variables (body temperature, general behaviour, appetite, and rate and quality of respiration) were monitored daily before and twice daily after challenge by personnel masked to treatment. Blinded necropsy was scheduled three days after infection (Day 8). Lungs were evaluated for lung consolidation (%) and lung-body weight ratios (%). Bacteriological examinations were performed on bronchial swabs and lung tissue (limit of quantification [LOQ] $10^3$ CFU/g).

Statistical analysis was performed by means of the software package SAS®. Treatment unit and statistical unit was the individual animal. The course of the clinical examination parameters was graphically displayed and statistically analysed. Treatment groups were compared pair-wise as contrasts in a repeated measures analysis of variance, with time and treatment-time-interaction as the within-subject effects and treatment as the between-subject effect. To ensure a multiple level of significance of $\alpha = 0.05$, the local level of significance for treatment effects was set to $\alpha = 0.005$ (Bonferroni adjustment). A comparison of treatment groups was carried out for the post mortem lung examination parameters. Pair-wise comparisons between treatment groups used a multiple-comparison procedure to detect significant differences. The Ryan-Einot-Gabriel-Welsch Multiple Range Test was used as the multiple-stage test which controlled the maximum experiment-wise error rate ($\alpha = 0.05$).

Clinical observations: The course of disease in animals treated with saline and Draxxin® reflects the severe challenge by *M. haemolytica* in this study. Five of the six animals from group 3 (saline) and one animal from group 2 (Draxxin®) died or were euthanized prior to scheduled necropsy for animal-welfare reasons whereas no animal died in group 1 (Zuprevo®) (Figure 2; Figure 3). General behaviour, appetite and respiration scores (Figures 4–6) indicated signs of clinical respiratory disease in groups 2 and 3 (Draxxin® and saline) after challenge until the end of the experimental phase; in contrast, scores in group 1 (Zuprevo®) peaked on the day of challenge or the next day and rapidly decreased to normal. Body temperature in group 1 (Zuprevo®) increased slightly after the challenge; in groups 2 and 3 (Draxxin® and saline)
the increase of body temperature was higher (Figure 7). Feed intake after infection was absent or reduced in groups 2 and 3 (Draxxin® and saline). In group 1 (Zuprevo®), the complete daily ration was consumed from one day after infection until the end of the study. Body temperature, general behaviour, appetite and respiration quality scores were significantly lower in group 1 (Zuprevo®) compared to groups 2 and 3 (Draxxin® and saline). These clinical variables were not significantly different between groups 2 and 3 (Draxxin® and saline).

Pathology: Figure 8 shows an example from group 3 (saline treated controls) of a lung severely affected by bronchopneumonia. Lung consolidation (Figure 9) and lung-body weight ratios (Figure 10) of group 1 (Zuprevo®) were significantly lower than those of groups 2 and 3 (Draxxin® and saline). No significant difference was detected between group 2 and 3 (Draxxin® and saline).

Bacteriology: In bronchial swabs taken at necropsy, no M. haemolytica growth was detected in any from the cattle treated with Zuprevo® (n = 0/6). Substantial growth of M. haemolytica was detected in bronchial swabs from all cattle in the saline-treated group 3 (n = 6/6) and all except for one animal in the Draxxin®-treated group 2 (n = 5/6) (Table 1).

In lung-tissue samples, growth of M. haemolytica was below the LOQ of 10⁵ CFU/g (n = 3/6) or comparatively low (n = 3/6, 10⁴ – 10⁵ CFU/g) in cattle treated with Zuprevo®. Substantially higher growth was observed in lung tissue of all animals in the saline-treated group 3 (n = 6/6; 10⁸ – 10¹⁰ CFU/g) and the Draxxin®-treated group 3 (n = 6/6; 10⁷ – 10⁹ CFU/g) (Table 1).

Clinical, pathological and bacteriological investigations showed that the preventive efficacy of Zuprevo® in this severe M. haemolytica challenge model of BRD was superior in comparison to saline-treated controls and Draxxin®. Preventive treatment with Zuprevo® provided superior reduction of pulmonary tissue bacterial counts. The reduction of M. haemolytica to below the LOQ in lung tissue in 50% of the animals and in bronchial fluid in 100% of the animals indicates bactericidal action of Zuprevo® (active ingredient tildipirosin) against M. haemolytica and confirms its long duration of action at high concentrations at the site of infection.

References:
Tables and Figures

Monitoring of general behaviour, body temperature, appetite, rate & quality of respiration*

Infection by intratracheal inoculation of ~1-2x10^9 colony forming units (CFU) M. haemolytica 7/2 Serotype A1 (tildipirosin MIC of 0.25 µg/mL)

* Personnel masked to treatment

**Figure 1.** Study Schedule

**Figure 2.** Mortality

**Figure 3.** Mortality (summary)

**Figure 4.** General behavior scores (means; Day 0–Day 8)

**Figure 5.** Appetite scores (means; Day 0–Day 8)
Score 0: normal; Score 1: slight dyspnea;  
Score 2: moderate dyspnea; Score 3: severe dyspnea  
Group 1 significantly different from Groups 2 and 3

Figure 6. Respiration quality scores (means; Day 0–Day 8)

Group 1 significantly different from Groups 2 and 3

Figure 7. Body temperature (means; Day 0–Day 8)

* Group 1 significantly different from Groups 2 and 3

Figure 8. Lung severely affected by bronchopneumonia  
(example from Group 3; saline treated controls)

* Group 1 significantly different from Groups 2 and 3

Figure 9. Lung consolidation (means; %)

Figure 10. Lung-body weight ratio (means; %)

Table 1. Bronchial-fluid and lung-tissue bacteriology

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Treatment</th>
<th>Growth of <em>M. haemolytica</em></th>
</tr>
</thead>
</table>
| **Bronchial fluid** | Group 1: Zuprevo® = (6/6)  
Group 2: Draxxin® +++ or ++++ (5/6)  
Group 3: Saline ++++ (6/6) |
| **Lung tissue**  | Group 1: Zuprevo® < LOQ (3/6); 10⁰–10⁷ (3/6)  
Group 2: Draxxin® 10⁷–10⁹ (6/6)  
Group 3: Saline 10⁹–10¹⁰ (6/6) |

* Bronchial fluid: “-“ no growth; “+++” pre-confluent growth;  
“++++” confluent growth  
Lung tissue: CFU/g; limit of quantification (LOQ) = 10⁰ CFU/g
4. Zuprevo®: Efficacy in the treatment of BRD in calves in comparison to Draxxin®

Kerstin E. Müller, Franziska Schmidt, Nadja Rohdich, Eva Zschiesche and Rainer Röpke
Zuprevo®: Efficacy in the treatment of BRD in calves in comparison to Draxxin®

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2 Intervet Innovation GmbH, a member of the MSD Animal Health Group

Abstract

Bovine Respiratory Disease (BRD) in calves is still a main problem in cattle production worldwide. The multifactorial etiology of BRD poses a challenge to farmers and veterinarians. Efficacious treatment of BRD based on adequate diagnostic tools is crucial to restore and maintain herd health. MSD Animal Health has recently been granted marketing authorization for Zuprevo® for the treatment and prevention of respiratory disease in cattle. Its active ingredient, tildipirosin, is the first tri-basic, 16-membered-ring macrolide. Tildipirosin is characterized by rapid absorption from the subcutaneous injection site and long-lasting high lung and bronchial fluid concentrations. These pharmacokinetic properties make Zuprevo® particularly suited for the treatment of BRD as demonstrated in a field trial in calves with BRD. In this study, Zuprevo® was efficacious and safe in the treatment of BRD over an observation period of 21 days. The onset of efficacy was most rapid with Zuprevo®. Overall, efficacy results were significantly non-inferior in comparison to Draxxin®.

Bovine Respiratory Disease Complex – etiology, diagnosis and treatment

The Bovine Respiratory Disease Complex (BRDC) is defined as a multifactorial disease of the respiratory tract resulting from complex interactions between environmental and host factors and pathogens (U.S. National Library of Medicine®). These factors act as stressors that adversely affect the immune system and other host defence mechanisms leading to an enhanced transmission of infectious agents. For calves several risk periods have been identified when the animals are prone to develop respiratory diseases: the neonatal period, the period around weaning and the period when maternal antibodies have vanished. Beef and veal calves, in addition, experience extra risk periods due to commingling of animals during the first ten days after arrival at the fattening farm and whenever regrouping of animals takes place. Besides these critical periods, numerous factors have been identified that contribute to outbreaks of BRDC. These include host factors (anatomical and functional weaknesses of the bovine respiratory tract, genetics), the macro- and the microclimate (temperature, humidity, air velocity, toxic gases, dust), management factors (management of the neonate, hygiene, nutrition, health status of the herd) and the presence of certain microorganisms (Figure 1). Among viruses of relevance for the BRDC, the Bovine Virus Diarrhoea Virus (BVDV), the Bovine Respiratory Syncytial Virus (BRSV) and the Bovine Herpesvirus -1 (BoHV-1) play a major role (Thiery, 2007). The same is true for bacteria as Mannheimia haemolytica, Pasteurella multocida, Bibersteinia trehalosi, Histophilus somni, Arcanobacterium pyogenes and mycoplasmata (M. bovis) (Griffin et al., 2010). Most microorganisms are normal inhabitants of the bovine upper respiratory tract. Due to certain strategies the latter bacteria are able to prevail over other bacteria and weaken the host’s defence mechanisms. Several techniques are available to sample the bovine respiratory tract in order to detect microorganisms participating in BRDC (Cooper and Brodersen, 2010). Less invasive techniques include obtaining nose swabs, sputum, tracheal swabs and blood samples. Trans-tracheal lavage, tracheobronchial lavage, bronchoalveolar lavage and lung biopsy are more invasive interventions. The procedure of (trans-) tracheal lavage is a suitable tool for the detection of bacteria species that participate in BRDC as the lower respiratory tract of healthy calves has been shown to be sterile.
Calves suffering from BRDC can be assigned to four different categories based on clinical findings (Reinhold, 2001): Grade I (subclinical) includes alert calves with normal breathing sounds at auscultation, Grade II (compensated) is characterized by the presence of symptoms of airway disease, a short period of fever and sustained appetite, Grade III (non-compensated) describes calves demonstrating dyspnoea, inappetence, enhanced or even bronchial breathing sounds and Grade IV (irreversible) includes calves exhibiting severe dyspnoea and anorexia and bronchial breathing sounds or even absent breathing sounds over extended areas of the lung field (Table 1). A treatment advice is linked to each of the four grades (Reinhold, 2001): Grade I calves do not need a specific treatment, Grade II demands an antibacterial treatment, Grade III a combination of antibiotics and anti-inflammatory drugs preferentially non-steroidal-anti-inflammatory drugs. Grade IV (irreversible) calves are unlikely to recover from BRDC due to the extended and irreversible damage to their lungs and for this reason should be euthanized (Table 1).

A Field Trial

Zuprevo® (active ingredient tildipirosin) is efficacious in the treatment of naturally acquired Bovine Respiratory Disease (BRD). Previously performed field studies had evaluated the primary efficacy endpoint up to 14 days after treatment; however, secondary efficacy parameters in those studies suggested an efficacy determined over a 21 days observation period.

This study was designed to confirm the efficacy of Zuprevo®, administered subcutaneously at a single dose of 4 mg tildipirosin/kg body weight (BW) in the treatment of BRD under field conditions over an observation period of 21 days. Efficacy was compared to the registered product Draxxin® (active ingredient tulathromycin, 2.5 mg/kg BW). The study was conducted in accordance with Good Clinical Practice, VICH GL9 (CVMP/VICH/595/98-FINAL).

Materials and methods

The study had an investigator-blinded, positive-controlled design and was conducted at one site in Germany. 104 calves (11 to 56 days old) were included in the study, 52 per treatment group. Animals showing the following signs of BRD on day 0 were included in the study (Table 2):

- Rectal temperature ≥ 40°C.
- Abnormal respiration (respiratory score ≥ 1).
- Abnormal general attitude (attitude score ≥ 1).

Animals were randomly allocated to treatment with either Zuprevo® (4 mg tildipirosin/kg BW) or Draxxin® (2.5 mg tulathromycin/kg BW). Treatments were administered as a single subcutaneous injection.

Clinical examinations were performed daily from day 0 until day 10 and on day 21. From day 0 to day 2, examinations were done three times daily (approximately 6 hours apart) and once daily from day 3 to day 10. Between day 11 and day 20, animals were observed daily for general health. Detailed examinations were performed in case of re-occurrence of BRD signs. Injection sites were observed before treatment and on days 1, 2, 3, 10, and 21 after treatment.

On day 0, all animals were sampled for bacteriological examination using the trans-tracheal lavage (TTL) technique. On day 0 and 21, animal weights were determined and information on potential concomitant viral and mycoplasma infections was gathered by obtaining paired serum samples from all animals. Haptoglobin concentrations were determined by photometric assay in serum samples taken on day 0, 1, 2, 3, 10, and 21. Between day 0 and day 21, feed intake was monitored.

Animals were regarded as treatment failures and withdrawn from the study if they fulfilled the following criteria:

- Between day 2 and day 10: rectal temperature ≥ 40°C and (respiratory score ≥ 2 or attitude score ≥ 2).
- Between day 11 and day 20: rectal temperature ≥ 40°C or respiratory score ≥ 2 or attitude score ≥ 1.
- On day 21: rectal temperature ≥ 40°C or respiratory score ≥ 1 or attitude score ≥ 1.

In case of animal withdrawal from the study between day 0 and day 21 due to BRD-related reasons (failure, late failure), these animals were re-sampled for bacteriological examination using the TTL technique on the day of withdrawal.

Necropsy was performed on 6 animals which completed the study successfully on day 21, 3 treated with Zuprevo® and 3 with Draxxin®. Lung tissue was sampled for bacteriological examination and lung lesion scores were determined.
Primary efficacy criterion was the treatment success rate on day 21, defined as the percentage of animals remaining in the study on day 21.

**Results**

Long term treatment success rates (treatment success on day 21) were 72.1 % (31 of 43) in the Zuprevo® group and 62.2 % (28 of 45) in the Draxxin® group (per-protocol [PP] population). Short term treatment success rates (intermediate treatment success on day 10) were 83.7 % (36 of 43) in the Zuprevo® group and 84.4 % (38 of 45) in the Draxxin® group (PP). Long term treatment failure on days 11 – 21 after short term (day 10) success (late failures) were 13.9 % (5 of 36) in the Zuprevo® group and 26.3 % (10 of 38) in the Draxxin® group. For all 3 criteria, Zuprevo® was non-inferior to Draxxin® (Table 3).

No mortality occurred in the Zuprevo® group, but 2 animals treated with Draxxin® died (1 in the PP, 1 in the intent-to-treat [ITT] population).

Rectal temperatures (Figure 3), respiratory and attitude scores (Figure 4), and respiration frequency decreased markedly in both treatment groups (PP) starting at day 1. Daily weight gains or lung scores did not differ significantly between groups (PP).

Before treatment, *Mannheimia (M.) haemolytica* (18.3 %), *Pasteurella (P.) multocida* (31.7 %), and *Histophilus (H.) somni* (1.0 %) were isolated by TTL (ITT) from the respiratory tract of the study animals. At withdrawal, distribution of isolates was as follows: 8.3 % *M. haemolytica*, 33.3 % *P. multocida*, and 0.0 % *H. somni* (ITT). *Mycoplasma (M.) bovis* was not isolated; however, 4 animals had concomitant *M. bovis* infections as detected by seroconversion (ITT). The minimum inhibitory concentrations (MIC) of tildipirosin were determined (Table 4).

Haptoglobin levels reflected the disease status in both treatment groups (data not shown). During the study, feed intake did not differ between both groups.

Mean onset of efficacy (i.e. first treatment effects as shown by rectal temperature < 39.5°C and attitude score = 0) was 26.8 h in the Zuprevo® group and 39.4 h in the Draxxin® group (PP) (Table 5).

No pain reaction was noted on injection in 86.5 % of the animals in the Zuprevo® group and in 65.4 % in the Draxxin® group (ITT). 9.6 % of animals in the Zuprevo® group and 13.5 % in the Draxxin® group had injection site reactions (ITT). 16 adverse events (concomitant diseases e.g. otitis, diarrhoea, lameness, omphalitis) were reported, 12 in the Zuprevo® group and 4 in the Draxxin® group. All except one (rated “unknown”) were unlikely related to the study medication (ITT).

**Conclusion**

Zuprevo® administered subcutaneously at a single dose of 4 mg/kg BW was efficacious and safe in the treatment of BRD within an observation period of 21 days. The onset of efficacy was most rapid with Zuprevo®. Overall, efficacy results were significantly non-inferior in comparison to Draxxin®.

**References:**

Tables and Figures

**Figure 1.** Etiology of BRD.

**Figure 2.** Trans-tracheal lavage (TTL) technique.

**Figure 3.** Course of rectal temperatures (PP population).

**Figure 4.** Attitude score (PP population).
Table 1. Severity of Bovine Respiratory Disease based on clinical symptoms and treatment advice (Reinhold 2001).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Subclinical (alert, normal breathing sounds)</td>
<td>No treatment</td>
</tr>
<tr>
<td>II</td>
<td>Compensated (symptoms of airway disease, appetite, short period of fever)</td>
<td>Antibiotics *</td>
</tr>
<tr>
<td>III</td>
<td>Non-compensated (dyspnoea, inappetence, enhanced or bronchial breathing sounds)</td>
<td>Antibiotics and NSAID *</td>
</tr>
<tr>
<td>IV</td>
<td>Irreversible (severe dyspnoea, anorexia, bronchial or even absent breathing sounds)</td>
<td>Euthanasia</td>
</tr>
</tbody>
</table>

* Bronchosecretolytica on indication

Table 2. Study schedule.

<table>
<thead>
<tr>
<th>Study day</th>
<th>Action</th>
<th>Sample</th>
<th>Evaluation injection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1–Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3–Day 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 11–Day 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2–Day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Serum sampling for determination of antibodies and haptoglobin
2 Serum sampling for determination of haptoglobin
3 Once per day
4 Animals that completed the study as treatment success

Table 3. Efficacy results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zuprevo*</th>
<th>Draxxin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term treatment success day 21 (Treatment success day 21)</td>
<td>72.1 % * (31 of 43)</td>
<td>62.2 % (28 of 45)</td>
</tr>
<tr>
<td>Short-term treatment success day 10 (Intermediate success day 10)</td>
<td>83.7 % * (36 of 43)</td>
<td>84.4 % (38 of 45)</td>
</tr>
<tr>
<td>Long-term failure (days 11–21) after short-term (day 10) success (Late failure day 11–21)</td>
<td>13.9 % * (5 of 36)</td>
<td>26.3 % (10 of 38)</td>
</tr>
</tbody>
</table>

*Significantly non-inferior to Draxxin*
Table 4. Minimum inhibitory concentrations (MICs) of tildipirosin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M. haemolytica (N=21)</th>
<th>P. multocida (N=42)</th>
<th>H. somni (N=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum MIC</td>
<td>0.25</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>Maximum MIC</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5. Onset of efficacy (PP population).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Onset of efficacy [h]*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Zuprevo® (N=31)</td>
<td>26.8</td>
</tr>
<tr>
<td>Draxxin® (N=28)</td>
<td>39.4</td>
</tr>
</tbody>
</table>

*First treatment effects as shown by rectal temperature < 39.5°C and attitude score = 0

Table 4. Minimum inhibitory concentrations (MICs) of tildipirosin.

Table 5. Onset of efficacy (PP population).

Notes

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