



# TECHNICAL BULLETIN

## Histophilosis: The compelling case for using Zuprevo™ in treatment of BRD caused by *Histophilus somni*

Histophilosis or *Histophilus somni*-associated disease is a common disease complex of North American cattle.

*H. somni* causes an acute, often fatal, septicemic disease that can involve the respiratory, cardiovascular, musculoskeletal or nervous systems either singly or together in a confined group of cattle. In cattle on pasture, occasional reproductive events are reported, particularly late-term abortions. Recently weaned calves are at higher risk of infection and death from histophilosis than previously weaned older calves, yearlings, or mature animals. The risk of infection with *H. somni* is highest early in the feeding period with "high-risk" calves in confinement establishing peak *H. somni* titers at about 21 to 23 days after arrival. The clinical disease is often seen earlier in the feeding period, but the average death is often 30 to 60 days on feed.<sup>1</sup>

*H. somni* is a gram-negative, non-motile, pleomorphic organism that prefers enriched broth under microaerophilic conditions for ideal growth. This makes it difficult to obtain isolates from typically collected nasal or deep pharyngeal swabs taken in the field and shipped to the lab. Samples need to be transported in special anaerobic media. Recent laboratory advances allow the use of PCR on nasal swabs and tissue samples. *H. somni* is considered a commensal organism of bovine mucous membranes, with both pathogenic and non-pathogenic strains being frequently found in the prepuce of bulls, the vagina of both cows and heifers and in the nasal passages<sup>2</sup>. The organism produces both an exotoxin and endotoxins. In an active pathogenic state, it avoids being killed by phagocytic cells, thus avoiding

an animal's immune response by residing as an intracellular organism in host cells. This characteristic also makes it more difficult to be reached by most antibiotics.

### Key Features of Zuprevo 18% (tildipirosin)<sup>3</sup>

- Zuprevo exhibits *in vitro* bacteriostatic and bactericidal action in bovine respiratory pathogens.
- Against *P. multocida* and *H. somni*, Zuprevo tends to be bacteriostatic, as the Minimal Bactericidal Concentration (MBC) generally is higher than the Minimum Inhibitory Concentration (MIC).
- Against *M. haemolytica*, Zuprevo is bactericidal, as the majority of isolates tested showed no difference in MIC versus MBC.
- The *in vitro* activity of tildipirosin is pH-dependent, showing highest activity at basic pH levels that are often found in diseased lung tissue.
- Tildipirosin produces a post-antibiotic effect (PAE) that varies between less than one hour and multiple hours depending on the bacterial species. The PAE is generally longer in the slow-growing, fastidious pathogen *H. somni* in comparison to both *M. haemolytica* and *P. multocida*.
- Against *H. somni*, Zuprevo has an MIC<sub>90</sub> of 4 ug/mL for both control and treatment of the organism.
- Zuprevo is passed into nasal secretions for at least 8 days after administration.

**IMPORTANT SAFETY INFORMATION: FOR USE IN ANIMALS ONLY. NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. TO AVOID ACCIDENTAL INJECTION, DO NOT USE IN AUTOMATICALLY POWERED SYRINGES WHICH HAVE NO ADDITIONAL PROTECTION SYSTEM. IN CASE OF HUMAN INJECTION, SEEK MEDICAL ADVICE IMMEDIATELY AND SHOW THE PACKAGE INSERT OR LABEL TO THE PHYSICIAN. DO NOT USE Zuprevo™ 18% IN SWINE.** Fatal adverse events have been reported following the use of tildipirosin in swine. **NOT FOR USE IN CHICKENS OR TURKEYS.** Cattle intended for human consumption must not be slaughtered within 21 days of the last treatment. Do not use in female dairy cattle 20 months of age or older. A withdrawal period has not been established in pre-ruminating calves. Do not use in calves to be processed for veal. The effects Zuprevo™ 18% on bovine reproductive performance, pregnancy and lactation have not been determined. Swelling and inflammation, which may be severe, may be seen at the injection site after administration. Subcutaneous injection may result in local tissue reactions which persist beyond slaughter withdrawal period.

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## Point 1: Zuprevo in lung tissue

Of all the antibiotics on the market in the United States, Zuprevo provides the highest level of drug in lung tissue for the longest period of time. Tissue levels above 5.0 µg/mL are found at 14 days after injection. This level is 20% above the 4 µg/ml MIC<sub>90</sub> for *H. somni*. Zuprevo given at the label dose of 4 mg/kg BW (1 mL/CWT), has therapeutic levels of the drug in lung tissue 2.25 times higher than the *H. somni* MIC<sub>90</sub> at 4 hours and 3.7 times at 24 hours after administration. By contrast, Draxxin<sup>®</sup> reaches its MIC<sub>90</sub> by 24 hours post-administration at label dose and falls below therapeutic levels shortly thereafter. In pneumonic lung, Draxxin fails to reach MIC<sub>90</sub> values at any time post-administration<sup>4</sup>.

## Point 2: Zuprevo Chemical Properties

Zuprevo is a 16-membered ring, tri-basic macrolide that inhibits protein synthesis of the bacterial ribosome. It is easily absorbed into tissue and bacterial cell walls but due to its large size is difficult to excrete from cells. *H. somni* is a fastidious, slow growing organism that is an ideal candidate for a drug like Zuprevo due to its ability to reach high tissue concentrations and long tissue activity level.

## Point 3: Is *H. somni* a problem in today's cattle populations?

*H. somni* is frequently isolated in lung, heart, brain and joint tissue from clinical cases. In a recent Kansas State Veterinary Diagnostic Laboratory report, over a three-year period *H. somni* was isolated from 16% to 27% of pneumonic lung samples submitted, with from 39% to 56% of those isolates found to have multi-drug resistance to three or more commonly used antibiotics. In the Lubbers report, nasopharyngeal swabs from cattle at arrival prior to antibiotic treatment found an average of 6.1% of over 440 swabs culture-positive for *H. somni*.<sup>5</sup> Similar results have been reported from diagnostic labs in Nebraska, Oklahoma and Iowa.<sup>6,7,8</sup>

## Point 4: Can Zuprevo control *H. somni* populations when used at arrival?

In a 2011 study of cattle purchased in Tennessee and transported to Nebraska for feeding, valuable information was obtained on the population dynamics of *H. somni* in high-risk cattle.<sup>9</sup> Cattle either received Zuprevo at arrival or a placebo. The study lasted for 42 days. Zuprevo significantly reduced sickness and improved performance over controls, specifically for *H. somni*. In the control group *H. somni* from nasal swabs continued to increase over the entire observational period (Figure 1), whereas in cattle receiving Zuprevo, *H. somni* levels remained significantly lower. This demonstrates that while *H. somni* increases in a population of animals over time, Zuprevo provides excellent control in holding down bacterial growth. This was also evident at necropsy, as none of the Zuprevo treated cattle had *H. somni* isolated from lung tissue.

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## Figure 1:

*Histophilus somni* growth patterns in feedlot cattle over a 42-day observation period with and without metaphylaxis.<sup>6</sup>

### Saline

Day	No. +	No. Swabs	%
-1	0	100	0%
0	0	100	0%
7	1	100	1%
14	7	98	7%
28	36	95	38%
42	50	94	53%
	94	587	16%

### Tildipirosin

No. +	No. Swabs	%
0	100	0%
0	100	0%
0	100	0%
0	98	0%
1	97	1%
12	97	12%
13	592	2%

## SUMMARY

Zuprevo is an antibiotic for rapid and sustained control or treatment of respiratory pathogens, particularly *H. somni*. Its established MIC<sub>90</sub> is well within lung tissue ranges for *M. haemolytica*, *P. multocida* and *H. somni*. For the respiratory pathogens *P. multocida* and *H. somni*, *in vitro* testing of tildipirosin indicates a tendency for bacteriostatic action. *In vitro* activity of tildipirosin is pH-dependent, showing highest activity at basic pH levels, which are pH levels frequently found in diseased bovine lung tissue, making it an excellent choice as a treatment product. Zuprevo is above the MIC<sub>90</sub> in lung tissue for over 14 days, which is superior to the therapeutic characteristics of Draxxin for *H. somni*.

## References

- <sup>1</sup>The Merck Manuals – Overview of Histophilosis – [www.merckmanuals.com/vet/generalized\\_conditions/histophilosis/overview\\_of\\_histophilosis](http://www.merckmanuals.com/vet/generalized_conditions/histophilosis/overview_of_histophilosis) on-line 05/29/13.
- <sup>2</sup>Perez, D.S. *et al.* *Histophilus somni*: Pathogenicity in Cattle. An Update. *An Vet (MURCIA)* 2010; 26: 5-21.
- <sup>3</sup>Zuprevo Monograph ZUPCA-17, 2012.
- <sup>4</sup>Pfizer FOI for Draxxin (tulathromycin) NADA 141-244.
- <sup>5</sup>A Diagnostic Lab Perspective on Respiratory Pathogens. Lubbers, 2013, data on file.
- <sup>6</sup>UNL Diagnostic Center News Spring 2013, Doster and Seelmeyer.
- <sup>7</sup>Lamm, C.G. *et al.* *J. Vet Diag Invest* 24(2): 277-282
- <sup>8</sup>ISU DX Lab Summary, data on file
- <sup>9</sup>Merck Study 11401-00. Data on file.

# ZUPREVO™ 18%



## (Tildipirosin)

### Injectable Solution for Cattle

#### ANTIMICROBIAL DRUG:

180 mg of tildipirosin/mL

For subcutaneous injection in beef and non-lactating dairy cattle only. Not for use in female dairy cattle 20 months of age or older or in calves to be processed for veal.

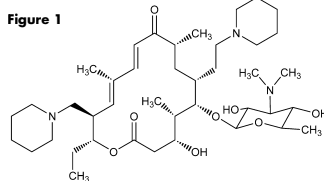
**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION:** Zuprevo™ 18% is a ready-to-use sterile injectable solution containing tildipirosin, a semi-synthetic macrolide antibiotic. Each mL of Zuprevo 18% contains 180 mg of tildipirosin as the free base, 82.5 mg citric acid monohydrate and 400 mg propylene glycol, and water qs with citric acid monohydrate added to adjust pH.

#### CHEMICAL NOMENCLATURE AND STRUCTURE:

Tildipirosin is the nonproprietary name for (11E,13E)-(4R,5S,6S,7R,9R,15R,16R)-6-(4-Dimethylamino-3,5-dihydroxy-6-methyl-tetrahydro-pyran-2-yloxy)-1,6-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-piperidin-1-yl-ethyl)-15-piperidin-1-ylmethyl-oxy-cyclohexadeca-11,13-diene-2,10-dione. The empirical formula is C<sub>41</sub>H<sub>71</sub>N<sub>3</sub>O<sub>8</sub>. The chemical structure of tildipirosin is shown below.

Figure 1



**INDICATIONS:** Zuprevo 18% is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in beef and non-lactating dairy cattle, and for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica*, *P. multocida*, and *H. somni*.

**DOSAGE AND ADMINISTRATION:** Inject subcutaneously as a single dose in the neck at a dosage of 4 mg/kg (1 mL/100 lb) body weight (BW). Do not inject more than 10 mL per injection site. Do not puncture the stopper of the respective vial size more than the tested number of punctures, shown in Table 1.

Clinical field studies indicate that administration of Zuprevo 18% (tildipirosin) Injectable Solution is effective for the control of respiratory disease in beef and non-lactating dairy cattle at "high risk" of developing BRD. Calves at high risk of developing BRD typically experience one or more of the following risk factors:

- Commingling from multiple sale barns/sources
- Extended transport times and shrink
- Exposure to wet or cold weather conditions or wide temperature swings
- Stressful arrival processing procedures (such as castration, dehorning, or branding)
- Recent weaning and poor vaccination history

Table 1 Number of punctures tested in the in-use study for the respective vial sizes

Vial size [mL]	Number of punctures tested in the in-use study
50	8
100	8
250	16

**WARNINGS: FOR USE IN ANIMALS ONLY. NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. TO AVOID ACCIDENTAL INJECTION, DO NOT USE IN AUTOMATICALLY POWERED SYRINGES WHICH HAVE NO ADDITIONAL PROTECTION SYSTEM. IN CASE OF HUMAN INJECTION, SEEK MEDICAL ADVICE IMMEDIATELY AND SHOW THE PACKAGE INSERT OR LABEL TO THE PHYSICIAN.**

Avoid direct contact with skin and eyes. If accidental eye exposure occurs, rinse eyes with clean water. If accidental skin exposure occurs, wash the skin immediately with soap and water. Tildipirosin may cause sensitization by skin contact.

For technical assistance or to report a suspected adverse reaction, call: 1-800-219-9286.

For customer service or to request a Material Safety Data Sheet (MSDS), call: 1-800-211-3573.

For additional Zuprevo 18% information go to [www.zuprevo.com](http://www.zuprevo.com).

For a complete listing of adverse reactions for Zuprevo 18% reported to CVM see:

<http://www.fda.gov/AnimalVeterinary/SafetyHealth>.

**DO NOT USE ZUPREVO 18% IN SWINE.** Fatal adverse events have been reported following the use of tildipirosin in swine. **NOT FOR USE IN CHICKENS OR TURKEYS.**

**RESIDUE WARNING:** Cattle intended for human consumption must not be slaughtered within 21 days of the last treatment.

Do not use in female dairy cattle 20 months of age or older. Use of this drug product in these cattle may cause milk residues. A withdrawal period has not been established in pre-ruminating calves. Do not use in calves to be processed for veal.

**PRECAUTIONS:** The effects of Zuprevo 18% on bovine reproductive performance, pregnancy and lactation have not been determined. Swelling and inflammation, which may be severe, may be seen at the injection site after administration. Subcutaneous injection may result in local tissue reactions which persist beyond the slaughter withdrawal period. This may result in trim loss of edible tissue at slaughter.

**CLINICAL PHARMACOLOGY:** Similar to other macrolides, tildipirosin inhibits essential bacterial protein biosynthesis with selective binding to ribosomal subunits in a bacteriostatic and time-dependent manner. Tildipirosin may be bactericidal against certain isolates of *M. haemolytica* and *P. multocida*.

The following plasma pharmacokinetic (PK) properties of tildipirosin have been observed following a subcutaneous injection at a dose of 4 mg/kg BW in the neck:

Table 2 Summary of pharmacokinetic characterization of tildipirosin administered subcutaneously to calves at a dose of 4 mg/kg BW.

Parameter	Average	SD
C <sub>max</sub> (ng/mL)	767*	284
T <sub>max</sub> (hr)	0.75*	0.43
AUC <sub>0-8hr</sub> (hr-ng/mL)	21017**	3499
AUC <sub>0-inf</sub> (hr-ng/mL)	24934**	3508
t <sub>1/2</sub> (hr)	210**	53

\* Value based on all 14 animals

\*\* Value based on 8 animals that were slaughtered at 504 hr post-treatment.

C<sub>max</sub>: Maximum observed plasma concentration

T<sub>max</sub>: Time at which C<sub>max</sub> was observed

AUC<sub>0-8hr</sub>: Area under the plasma concentration versus time curve measured from time zero to the last sample with tildipirosin concentrations exceeding the limit of quantification of the analytical method

AUC<sub>0-inf</sub>: AUC estimated from time zero to time infinity t<sub>1/2</sub>: Terminal elimination half life

Due to the extensive partitioning of macrolides into tissues and because of their multi-fold greater concentrations in bronchial fluid relative to that observed in the blood, plasma drug concentrations underestimate concentrations at the site of action<sup>1</sup>. This is shown for tildipirosin in the following table, where bronchial fluid samples were collected in live, healthy calves, and compared to the concentrations in plasma observed in these same animals:

Table 3 Bronchial fluid-to-plasma ratio of tildipirosin in non-anesthetized cattle following a subcutaneous injection at a dose of 4 mg/kg BW in the neck

Time (hours)	Bronchial fluid (BF) concentration (ng/g)		Plasma (P) concentration (ng/mL)		BF/P Ratio
	Average	SD	Average	SD	
4	1543	895	297	81.8	5.20
10	2975	1279	242	96.7	12.3
24	3448	1433	136	53.9	25.4
72	3489	1712	70.7	29.0	49.3
96	1644	2024	60.2	29.0	27.3
120	1619	1629	52.3	19.9	30.9
240	1937	1416	27.1	10.8	71.5
336	1225	1682	26.1	9.2	47.0
504	935	1032	16.8	1.7	55.6

Tildipirosin concentrations in bronchial fluid collected *in vivo* from non-anesthetized cattle reflect the bacterial exposure to drug concentrations at the site of action.

<sup>1</sup>Nightingale, C.H. (1997) Pharmacokinetics and pharmacodynamics of newer macrolides. The Pediatric Infectious Disease Journal, 16, 438-443.

**MICROBIOLOGY:** Tildipirosin has shown *in vitro* and *in vivo* antibacterial activity against the bacteria *M. haemolytica*, *P. multocida*, and *H. somni*, three pathogens associated with BRD.

The minimum inhibitory concentrations (MICs) of tildipirosin against the indicated BRD pathogens were determined using the methods described in the M31-A2 standard of the Clinical and Laboratory Standards Institute (CLSI) and are shown in Table 4.

The MICs of tildipirosin were determined for isolates of *M. haemolytica*, *P. multocida*, and *H. somni* obtained from two BRD field studies. In both studies, tested isolates of *M. haemolytica* and *P. multocida* were obtained from nasopharyngeal swabs taken prior to treatment from all study animals. Tested isolates of *H. somni* were obtained from nasopharyngeal swabs taken prior to treatment from all study animals and from nasopharyngeal swabs taken from saline-treated animals classified as treatment failures.

Table 4 Tildipirosin minimum inhibitory concentration (MIC) values\* of indicated pathogens isolated from BRD field studies in the U.S.

Indicated Pathogens	Year of isolation	Study	Number of isolates	MIC50** (µg/mL)	MIC90** (µg/mL)	MIC range (µg/mL)
<i>Mannheimia haemolytica</i>	2007	Treatment	484	1	2	0.25 to >32
	2007 to 2008	Control	178	1	1	0.25 to >32
<i>Pasteurella multocida</i>	2007	Treatment	235	0.5	1	0.12 to >32
	2007 to 2008	Control	273	0.5	1	≤0.03 to 4
<i>Histophilus somni</i>	2007	Treatment	33	2	4	1 to 4
	2007 to 2008	Control	32	2	4	1 to >32

\* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.

\*\* The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

**EFFECTIVENESS:** In a multi-location field study, calves with naturally occurring BRD were treated with tildipirosin. The treatment success rate of the tildipirosin-treated group was compared to the treatment success rate in the saline-treated control group. A treatment success was defined as a calf not designated as a treatment failure from Day 1 to 13 and with normal attitude, normal respiration, and a rectal temperature of <104°F on Day 14. The treatment success rate was significantly higher (p=0.003) for the tildipirosin-treated group (229/300, 76%) compared to the saline-treated control group (96/200, 32%). There were no BRD-related deaths in the tildipirosin-treated group compared to a 7% (21/300) BRD-related mortality rate in the saline-treated group.

In another multi-location field study, calves at high risk for developing BRD were administered tildipirosin. The treatment success rate of the tildipirosin-treated group was compared to the treatment success rate in the saline-treated control group. A treatment success was defined as a calf not designated as a treatment failure based on clinical respiratory and attitude scoring and, if necessary, rectal temperature measurement of <104°F through the end of the study (Day 14). The treatment success rate was significantly higher (p=0.0001) for the tildipirosin-treated group (305/386, 79%) compared to the saline-treated group (197/387, 51%). There were three BRD-related deaths during the study (one tildipirosin-treated calf and two saline treated calves).

**ANIMAL SAFETY:** A target animal safety study was conducted using Zuprevo 18% administered in 5-month-old cattle as three subcutaneous doses of 4, 12, or 20 mg/kg BW given 7 days apart (1X, 3X, and 5X the labeled dose). Animals remained clinically healthy during the study at the labeled dose. Injection site swelling and inflammation, initially severe in some animals, was observed that persisted to the last day of observation (21 days after injection). No other drug-related lesions were observed macroscopically or microscopically at the labeled dose.

A separate injection site tolerance study was conducted using Zuprevo 18% in 5- to 9-month-old cattle administered as a single subcutaneous injection of 10 mL. Injection site swelling and inflammation, initially severe in some animals, was observed that persisted to the last day of observation (35 days after injection). No other drug-related clinical signs were observed.

**STORAGE CONDITIONS:** Do not store above 30°C (86°F). Do not freeze. The maximum storage time after first puncture is 28 days at or below 25°C (77°F).

**HOW SUPPLIED:** Zuprevo 18% is supplied in 50, 100 and 250 mL, amber glass, sterile, multi-dose vials.

U. S. Patent: 6,514,946

NADA 141-334, Approved by FDA

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