ZUPREVO™ (tildipirosin) You're in control

TECHNICAL BULLETIN

Determination of the amount of pulmonary lesions when cattle are challenged with *Mannheimia haemolytica* 10 days after administration of either tildipirosin (Zuprevo™) or tulathromycin (Draxxin®)[1,2]

Introduction

The objective of this project was to evaluate the long-term efficacy of two antimicrobials (Zuprevo and Draxxin) on mitigation of pulmonary lesions in calves challenged with *M. haemolytica* 10 days post-treatment. All study procedures were conducted in accordance with a protocol (#3198) approved by the Kansas State University Institutional Animal Care and Use Committee.

Key Points

This Technical Service Bulletin is derived from the first peer-reviewed, published article showing greater reduction in lung lesions by Zuprevo than Draxxin.[3]

In total, these findings illustrate that following a *M. haemolytica* challenge 10 days post-administration of saline (SAL), Draxxin (DRX), or Zuprevo (ZUP), the ZUP calves had less severe pneumonia than the SAL or DRX calves.

ZUP demonstrated bactericidal activity and statistically significant superiority of DRX and SAL in clinical illness scores and pulmonary lesions.

Materials and Methods

Test animals were 38 local auction barn-derived calves, with an average weight of 391 lb. The day of arrival at the feeding facility (Day 0), five calves were reserved as replacements for any calves that became ill prior to initiation of the study. The remaining 33 calves were randomly assigned to one of three replicates, and treatments were administered.

Group 1	12 head	Zuprevo (ZUP) treatment	4.0 mg per kg body weight
Group 2	12 head	Draxxin (DRX) treatment	2.5 mg per kg body weight
Group 3	9 head	Saline (SAL) controls	0.02 mL per kg body weight

10 days post-treatment, calves were administered an endoscopically guided *M. haemolytica* challenge to the first bronchus accessory lobe.

Three days post-challenge, calves were harvested. Primary outcomes of interest were percent lung lesions, lung-to-body weight ratios, and clinical illness scores (CIS).

Project Timeline

Treatment → **Challenge** → **Harvest**

Day 0 → 10 days post-treatment → 3 days post-challenge

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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[2]Derek Mosier; Department of Diagnostic Medicine and Pathobiology, Kansas State University, 1600 Denison Ave. Manhattan, Kansas 66502.
[3]Armine, David E. *et al.* November 15, 2013. Pulmonary lesions and clinical disease response to *Mannheimia haemolytica* challenge 10 days following administration of tildipirosin or tulathromycin. *J. Anim Sci.* jas. 2013-6577.

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Results

For the reader's convenience and ease of understanding, results will be presented primarily in graph format, with an appendix of lung pathology photos for further clarification of product efficacy. First a summary of significant P values that may not be further addressed in the text:

Clinical Signs Post-Challenge	ZUP<DRX & SAL	P<0.05
Respiratory Rate Scores Post-Challenge	ZUP & DRX<SAL	P<0.05
Lung Lesions (overall percentages of lung consolidation)	ZUP<DRX & SAL	P<0.05
Lung Percent of Body Weight	ZUP<DRX & SAL	P<0.05

Figure 1: Clinical Signs Post-Challenge

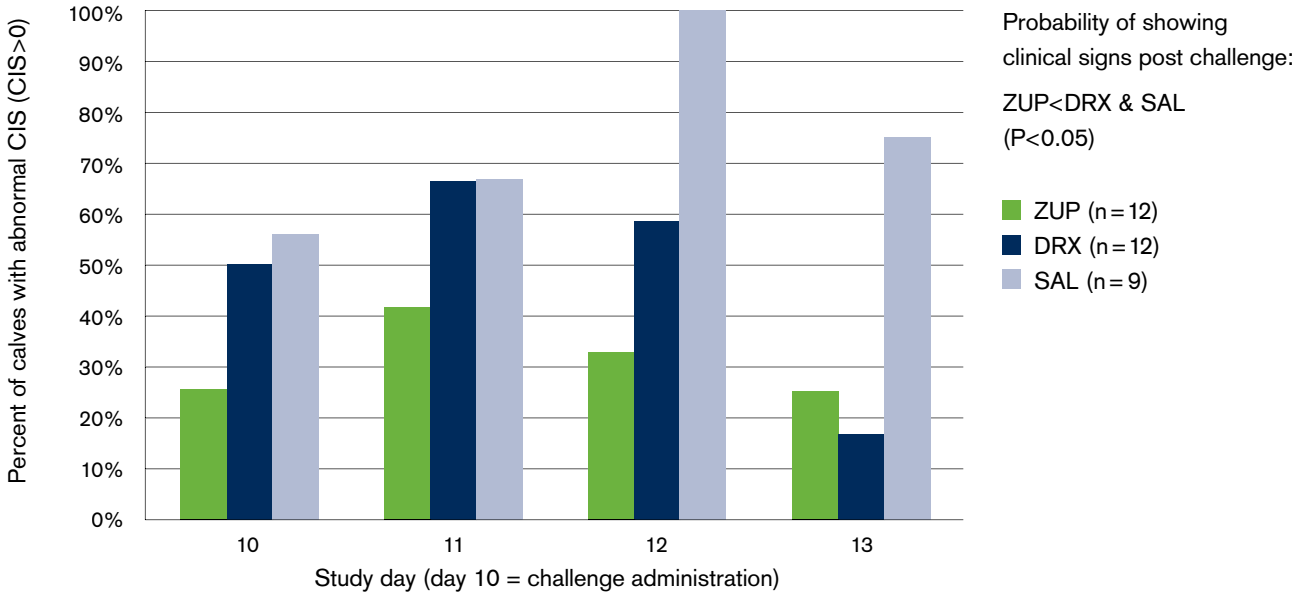


Figure 2: Respiratory Rate Scores Post-Challenge

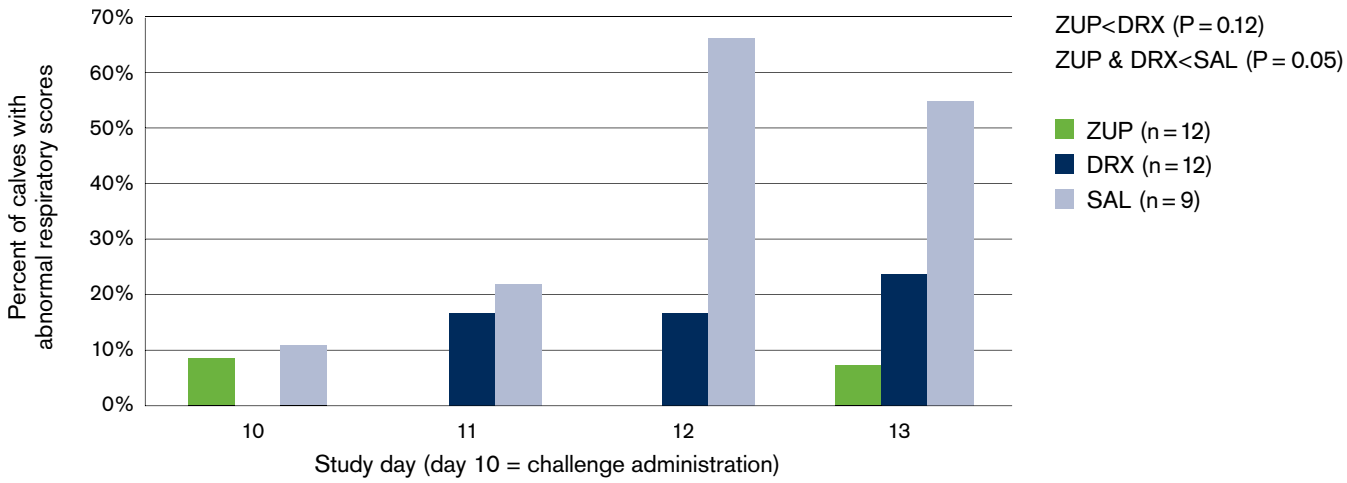
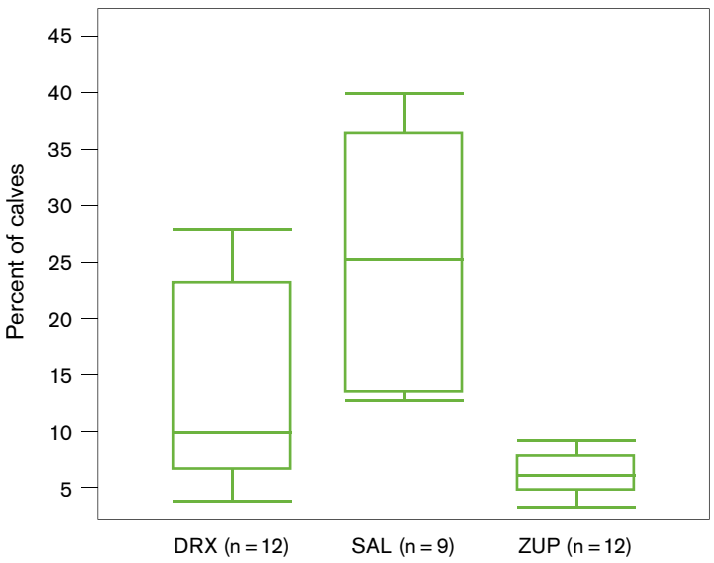


Figure 3: Lung Lesions

	Lung lesions, all lobes			Lung lesions, cranio-ventral lobes only		
Pulmonary consolidation score	**ZUP**	**DRX**	**SAL**	**ZUP**	**DRX**	**SAL**
Average	8.7	13.1	25.5	21.4	33.4	57.4
Median	5.9	9.9	25.1	16.4	27.9	61.2
Standard deviation	9.8	8.6	11.2	18.4	18.9	23.8
Minimum	3.3	3.8	12.8	9.42	10.9	25.2
Maximum	39.1	27.8	39.8	77.8	68.6	92.7

Figure 4: Variability of Full Percent Lung Lesions by Treatment Group



Average collective scores per treatment group:

ZUP = 8.7 DRX = 13.1
SAL = 25.5 ZUP<DRX & SAL (P<0.05)

Treatment results: Bacteriology

A major difference was observed between Zuprevo and Draxxin, with *M. haemolytica* recovered in 100% (12/12) of calves' lungs from Draxxin-treated calves, the same as in saline control calves. *M. haemolytica* was recovered from 25% (3/12) of Zuprevo-treated calves.

Discussion and Conclusion

Since the percentage of pulmonary lesion is an estimated value, another more precise quantitative measurement to determine the amount of pulmonary damage is the total lung weight as a percent of the animal's body weight. In this study, the pulmonary percent of body weight agreed with the overall level of lung lesions for calves in the SAL and DRX groups, with both groups having higher lung percent of body weight compared to ZUP calves.

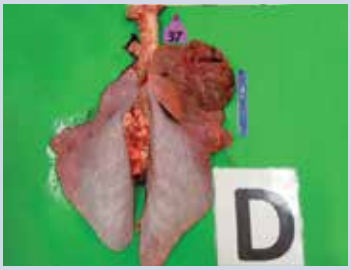
The probability of calves in the DRX group receiving abnormal clinical scores was higher than ZUP-treated calves in the CIS and appetite. Calves in the SAL-treated group had the highest probabilities of all groups for receiving abnormal scores. These clinical observations agree with the necropsy findings related to the level of pulmonary lesions in each group.

In total, these findings illustrate that following an *M. haemolytica* challenge 10 days post-administration of SAL, DRX, or ZUP, the ZUP calves had less severe pneumonia than the SAL or DRX calves; ZUP demonstrated bactericidal activity and statistically significant superiority over Draxxin and saline in clinical illness scores and pulmonary lesions; and this Technical Service Bulletin is derived from the first peer-reviewed, published article showing greater reduction in lung lesions by Zuprevo than Draxxin.[3]

Appendix, Lung Pathology Photos

Slide identity is—left to right—calf number, treatment product abbreviation, lung lesion score using all lung lobes; and lung lesion score using cranio-ventral lobes only.

Saline Treated Calves

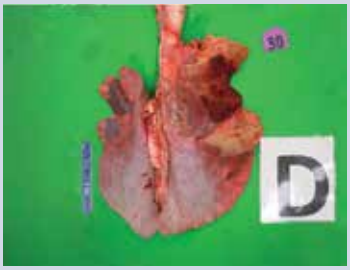


#37, SAL, 12.82%, 35.06%

35, SAL, 39.80%, 76.92%

#16, SAL, 34.43%, 74.42%

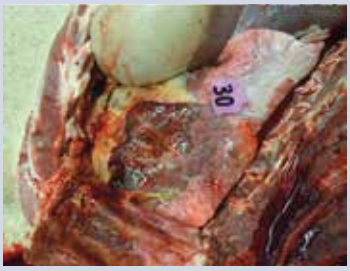
Draxxin Treated Calves



#30, DRX, 27.8%, 48.8%

#10, DRX, 6.3%, 18.2%

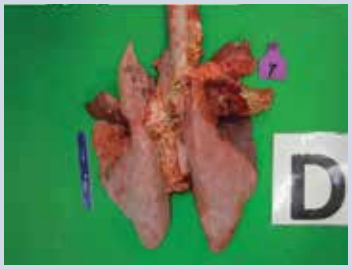
#18, DRX, 26.4%, 68.6%



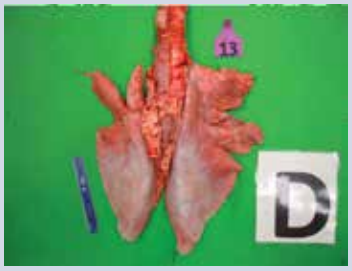
#30, DRX, 27.8%, 48.8%

Zuprevo Treated Calves

#9, ZUP, 3.6%, 9.4%



#7, ZUP, 39.1%, 77.8%



#13, ZUP, 3.3%, 9.5%

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ZUPREVO™ 18%

(Tildipirosin)

Injectable Solution for Cattle

ANTIMICROBIAL DRUG:
180 mg of tildipirosin/mL

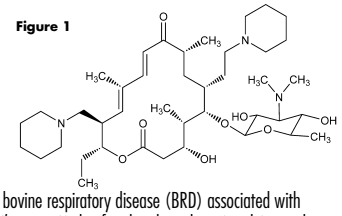
For subcutaneous injection in beef and non-lactating dairy cattle only. Not for use in female dairy cattle 20 months of age or older or in calves to be processed for veal.

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 Zuprevo18% 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)-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-piperidin-1-yl-ethyl)-15-piperidin-1-ylmethyl-oxacyclohexadeca-11,13-diene-2,10-dione. The empirical formula is $C_{41}H_{71}N_3O_8$. 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 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.
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_{max} (ng/mL)	767*	284
T_{max} (hr)	0.75*	0.43
$AUC_{0\text{-}last}$ (hr·ng/mL)	21017**	3499
$AUC_{0\text{-}inf}$ (hr·ng/mL)	24934**	3508
$t_{1/2}$ (hr)	210**	53

* Value based on all 14 animals
** Value based on 8 animals that were slaughtered at 504 hr post-treatment.
C_{max}: Maximum observed plasma concentration
T_{max}: Time at which Cmax was observed

$AUC_{0\text{-}last}$: 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

$AUC_{0\text{-}inf}$: AUC estimated from time zero to time infinity $t_{½}$: 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[1]. 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.

[1]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)
Mannheimia haemolytica	2007	Treatment	484	1	2	0.25 to >32
	2007 to 2008	Control	178	1	1	0.25 to >32
Pasteurella multocida	2007	Treatment	235	0.5	1	0.12 to >32
	2007 to 2008	Control	273	0.5	1	≤0.03 to 4
Histophilus somni	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
Use Only as Directed

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