A Placebo Controlled Field Efficacy Study of a Novel Trimethoprim and Sulfadiazine Oral Suspension in Horses with Lower Respiratory Tract Infections caused by *Streptococcus equi* subsp. *zooepidemicus*

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EQUISUL-SDT®
(Sulfadiazine/Trimethoprim)
Antimicrobial
Oral suspension
400 mg/mL

Aurora Pharmaceutical, LLC
Investigators

- Investigators who contributed cases
  - Scott R. McClure, DVM, PhD, DACVS
  - Craig R. Reinemeyer, DVM, PhD
  - Breck D. Hunsaker, DVM, PhD
  - Gary W. White, DVM
Introduction

* Potentiated sulfonamides
  * Sulfadiazine, Sulfamethazine, Sulfamethoxazole
    * Inhibit folate synthesis by antagonism of para-aminobenzoic acid which inhibits DNA synthesis

* Pyrimidines
  * Trimethoprim
    * Inhibit folate synthesis by binding dihydrofolate reductase

* Synergistic interruptions in folate synthesis
  * Decrease antimicrobial resistance
  * Provide bactericidal activity at lower MIC
Potentiated Sulfas

- Routinely used in equine practice
  - One of few oral antimicrobials available
  - Broad spectrum bactericidal activity
  - Typically a 1:5 ratio of trimethoprim:sulfa

- EQUISUL-SDT®
  - TMP:SDZ
Simplified pathway for the action of trimethoprim-sulfonamide combinations.

Veterinary Pharmacology & Therapeutics, 9th edition, pg 838.
Streptococcus = Strep.

**Strep. zoo.**
- Bacteria, Gram-positive cocci
- Lower respiratory disease
- *Strep. equi* subspecies *zooepidemicus*
  - *Strep. equi-zoo*
  - *Strep. zoo.*

**Strep. equi.**
- Bacteria, Gram-positive cocci
- Strangles
- Don’t confuse
- *Strep. equi* subspecies *equi*
  - *Strep. equi*
Strep. zoo.

* One of the most common bacterial pathogens in horses
* Routinely isolated from the respiratory tract
* May also be isolated from wounds and the urogenital tract
* Often opportunistic when normal defenses are compromised
MIC
Minimum Inhibitory Concentration

* MIC₉₀ for Strep. zoo.
  * 0.12 mcg/ml TMP and 2.38 mcg/ml SDZ
    * Bade et al. Vet Therapeutics. 2009
* Pilot efficacy study: TMP/SDZ suspension
  * MIC₉₀ 0.12 / 2.4 mcg/ml
* EQUISUL-SDT product dose 24mg/kg
  * MIC₉₀ SDZ 100% and TMP 98%
Evaluate effectiveness of a novel trimethoprim and sulfadiazine oral suspension in naturally occurring Strep. zoo. Pneumonia in horses under field use conditions
Materials and Methods

* Multicenter
  * 5 sites
* Randomized
* Blinded
* Placebo controlled
* Naturally occurring bronchopneumonia
Materials and Methods

- Investigator owned horses
- > 1 year of age
- Naturally occurring bronchopneumonia
  - Purchased
  - Comingled
  - Transported

- Enrollment criteria
  - Rectal Temp
    - ≥ 101°F
  - Respiratory rate
    - >24 breath/minute
  - Auscultation
    - Abnormal sounds
  - Intermittent, spontaneous cough
Exclusion Criteria

* Pregnancy
* Lactation
* < 1 year old
* Previous treatment for respiratory disease
* Liver disease
* Strangles
* Refractory to handling (Meaner than snakes)
Materials and Methods

* Transtracheal wash: TTW
  * Enrollment and removal if not rescued
  * Identify predominant equine pathogens
  * Quantify *Strep. zoo.*
TTW

Flush

60ml Saline
Materials and Methods

* **Clinical Exams**
  * Days 0, 5, 10, 17
  * Evaluated in same model with treatment group, time and group by time interactions

* **Daily observations**
  * Twice daily
  * Through Day 17
  * Included specific evaluation of health and fecal consistency

* **Clinical pathology (blood)**
  * CBC, Serum chemistry, Clotting profile
  * Day 0 (enrollment)
  * Withdrawal from study
    * Treatment failure
  * Day 17 (completion)
# Clinical Scores

<table>
<thead>
<tr>
<th>Score</th>
<th>Rectal Temperature</th>
<th>Respiratory Rate</th>
<th>Respiratory Sounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 101 F</td>
<td>≤24 Breaths/min</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>≥101 F</td>
<td>&gt;24 Breaths/min</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Coughing</th>
<th>Fecal Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None elicited by laryngeal palpation</td>
<td>Formed</td>
</tr>
<tr>
<td>1</td>
<td>Cough elicited by laryngeal palpation</td>
<td>Mixed pellets and liquid</td>
</tr>
<tr>
<td>2</td>
<td>Intermittent spontaneous</td>
<td>Unformed “cowpie”</td>
</tr>
<tr>
<td>3</td>
<td>Sustained without stimulus</td>
<td>Watery Diarrhea</td>
</tr>
</tbody>
</table>
* **EQUISUL-SDT**
  * TMP/SDZ suspension
  * 67mg TMP and 333mg SDZ per ml = 400mg/ml
  * 24mg/kg body weight combined active to nearest ml
  * Orally
  * Twice daily for 20 treatments

* **Placebo**
  * Saline, equivalent volume and timing
Success versus Failure

Clinical improvement
* Improvement at Day 5
  * Temp down 2°F or <101°F
  * Respiratory rate <24 breath/min
* Normal at Day 10
* No relapse at Day 17
* Any horse NOT meeting these criteria was considered a treatment failure

Primary outcome variable
* Success at Day 17
  * Clinical response to treatment
  * Evaluated in a generalized linear mixed model
Day 17 negative for *Strep. zoo.*

Day 0, TTW, *Strep. zoo.* scores
- ≥3 in pure culture
- 4 in mixed culture

Fisher’s exact test used to compare clinical success between treatment and control groups
Adverse Event Evaluation

- Every horse enrolled regardless of outcome
  - Daily observations
    - General health
    - Attitude
    - Appetite
    - Fecal consistency
      - Diarrhea
  - Statistical analysis
    - $P$ value 0.10
    - Avoid missing significance
Blinding

* **Horses blocked**
  * By site
  * 2:1 Treatment: Placebo
    * Randomized schedule
* **Observations**
  * Observers did NOT take part in treatment
  * Observations not less than 1 hour post treatment
  * Horses were cleaned of any treatment residue
Success Criteria

Treatment Success

Label claim/primary outcome

* Clinical response to treatment
  * Rectal temperature
    * 2°F below Day 0 or <101°F by Day 5; and <101°F on subsequent exams
  * Respiratory rate
    * ≤24 breath/min
  * Normal auscultation
  * Intermittent cough
    * Days 5 and 10
  * No cough
    * Day 17

Treatment Effectiveness

Microbiological success

* Eradication of Strep. zoo.
* Horses enrolled and treatment begun before bacterial status was known
* Semiquantitative colony count (Strep. zoo.)
  * Pure culture
    * 101-1000 colonies
  * Mixed culture
    * 1000 colonies
* Day 17 culture negative for Strep. zoo.
Study Animal Metrics

- 270 cases enrolled
- 110 QHs
- 37 Paints
- 24 Tenn Walkers
- 9 Paso Fino
- 7 total App, MoFo, Trotters, Arabian
- 34 mixed
- 49 grade

- Age
  - Mean 3 yrs.
  - Range 1 to 25 yrs.

- Gender
  - 148 females
  - 57 males
  - 65 geldings
Evaluable Horses

182 received EQUISUL-SDT
88 received placebo

112 treated evaluated for primary outcome
61 placebo evaluated for primary outcome
112 receiving treatment and evaluated for clinical effectiveness (66/112)  
* 59% considered successes  
61 receiving placebo and evaluated for clinical effectiveness (9/61)  
* 15% considered successes  

P = 0.0123
Failures fail fast

- Day 5
  - 41% (25/61) Control
  - 6.3% (1/112) EQUISUL

- Day 10
  - 49% (30/61) Control
  - 11% (12/112) EQUISUL

- Indication of a good disease challenge
Overall Strep. zoo. Recovery

- Strep. zoo.
  - Recovered from 300/485 TTWs
  - 62% of all TTW submitted
- Indication of a good disease presence

- Mean MIC$_{90}$ (mcg/mL)
  - Expected:
    - 0.25/4.75 (TMP/SDZ)
  - Actual:
    - 112/300 (37.3%)
    - MIC$_{90}$ 0.12/2.4 (TMP/SDZ)
    - 185/300 (61.7%)
    - MIC$_{90}$ 0.25/4.75 (TMP/SDZ)
Strep. zoo. Elimination

* Treatment effectiveness
  * Strep. zoo. Elimination
    * 66% of EQUISUL
      * 76/116
    * 21% of Control spontaneous
      * 12/56

![Strep. zoo. Elimination Chart]
## Mean Clinical Scores

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>Treatment</th>
<th>Day 5 Mean</th>
<th>Day 10 Mean</th>
<th>Day 17 Mean</th>
<th>Overall Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temp (°F)</td>
<td>EQUISUL-SDT</td>
<td>100.0</td>
<td>99.5</td>
<td>99.7</td>
<td>99.8</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>101.2</td>
<td>100.1</td>
<td>99.9</td>
<td>100.6</td>
<td></td>
</tr>
<tr>
<td>Respiratory rate (breath/min)</td>
<td>EQUISUL-SDT</td>
<td>23.4</td>
<td>21.8</td>
<td>20.4</td>
<td>21.8</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>29.0</td>
<td>23.2</td>
<td>24.5</td>
<td>25.2</td>
<td></td>
</tr>
<tr>
<td>Auscultation score</td>
<td>EQUISUL-SDT</td>
<td>1.8</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.9</td>
<td>1.7</td>
<td>1.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Coughing score</td>
<td>EQUISUL-SDT</td>
<td>0.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.3</td>
<td>1.0</td>
<td>0.7</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>
Mean Clinical Scores
Rectal Temperature and Respiratory Rate

Rectal Temperature
- EQUISUL
- Control

Respiratory Rate
- EQUISUL
- Control

[Graphs showing temperature and respiratory rate over different days and overall.]
Mean Clinical Scores
Auscultation Score and Coughing Score

Auscultation Score
- EQUISUL
- Control

Coughing Score
- EQUISUL
- Control

Bar charts showing scores for Auscultation and Coughing over days 5, 10, 17, and overall.
## Adverse Events Summary

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>EQUISUL-SDT (n = 182)</th>
<th>Control (n = 88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colic</td>
<td>1.6% (3)</td>
<td>2.3% (2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1.1% (2)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Abscess/swelling</td>
<td>1.1% (2)</td>
<td>1.1% (1)</td>
</tr>
<tr>
<td>Lameness</td>
<td>0.5% (1)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Swollen lymph nodes</td>
<td>0.5% (1)</td>
<td>1.1% (1)</td>
</tr>
<tr>
<td>Strangles</td>
<td>0.5% (1)</td>
<td>1.1% (1)</td>
</tr>
<tr>
<td>Abortion</td>
<td>0% (0)</td>
<td>1.1% (1)</td>
</tr>
<tr>
<td>Swollen muzzle</td>
<td>0% (0)</td>
<td>1.1% (1)</td>
</tr>
</tbody>
</table>
Fecal Scoring
Fecal consistency scores between groups no difference

<table>
<thead>
<tr>
<th>Reaction</th>
<th>EQUISUL-SDT (n=182)</th>
<th>Control (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loose stool (including diarrhea)</td>
<td>69 (38%) (at least one episode)</td>
<td>29 (33%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (1.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Colic</td>
<td>3 (1.6%)</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Parameter reference range (Mean)</td>
<td>EQUISUL-SDT</td>
<td>Control</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>Absolute Lymphocytes 1.4 to 8.8 x 10^3/μL</td>
<td>3.58</td>
<td>3.16</td>
</tr>
<tr>
<td>Relative Lymphocytes 25 to 70%</td>
<td>33.08</td>
<td>26.86</td>
</tr>
<tr>
<td>Absolute Monocytes 0.0 to 0.9 x 10^3/μL</td>
<td>0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>Relative Monocytes 0.0 to 7.0%</td>
<td>4.57</td>
<td>5.45</td>
</tr>
<tr>
<td>Absolute Neutrophils 1.7 to 8.1 x 10^3/μL</td>
<td>6.36</td>
<td>7.63</td>
</tr>
<tr>
<td>Relative neutrophils 30 to 65%</td>
<td>56.59</td>
<td>62.65</td>
</tr>
<tr>
<td>Hematocrit 32–52%</td>
<td>32.37</td>
<td>30.95</td>
</tr>
<tr>
<td>Mean corpuscular volume 34–58 fL</td>
<td>43.67</td>
<td>43.26</td>
</tr>
<tr>
<td>Fibrinogen 100–400 mg/dL</td>
<td>300.5</td>
<td>372.9</td>
</tr>
<tr>
<td>Prothrombin time 10.4–12.2 sec</td>
<td>11.58</td>
<td>11.96</td>
</tr>
<tr>
<td>Albumin 2.7–4.2 g/dL</td>
<td>3.08</td>
<td>2.98</td>
</tr>
<tr>
<td>Globulin 3.0–5.1 g/dL</td>
<td>4.31</td>
<td>4.47</td>
</tr>
<tr>
<td>A/G ratio 0.7–1.5</td>
<td>0.75</td>
<td>0.69</td>
</tr>
<tr>
<td>Urea nitrogen 8–25 mg/dL</td>
<td>19.46</td>
<td>17.53</td>
</tr>
<tr>
<td>Calcium 11.2–13.6 mg/dL</td>
<td>11.32</td>
<td>10.98</td>
</tr>
<tr>
<td>Potassium 3.0–5.1 mmol/dL</td>
<td>4.41</td>
<td>4.27</td>
</tr>
<tr>
<td>Sodium 132–146 mmol/L</td>
<td>135.2</td>
<td>134.3</td>
</tr>
</tbody>
</table>

**Clinical Pathology**

There were no clinically significant changes in clinical pathology parameters related to administration of EQUISUL-SDT.

Small differences seen are likely biologic variation or associated with improved feed intake among treated horses.
EQUISUL-SDT Study Summary

- 24 mg/kg (11mg/lb) body weight
- Administered twice daily
- 10 days (20 doses)
- Significantly improved clinical success in the treatment of horses with naturally occurring lower respiratory pneumonia

- The criteria for treatment success or failure were time-point dependent
- Required improvement by Day 5
- Once considered a failure, the animal was left in the failure category even if it improved
EQUISUL-SDT
Study Summary

* Potential for gastrointestinal flora disturbance (diarrhea), but, no more than horses receiving other antimicrobials (Wilson et al JVIM 1996)
* No problems seen in this study

* There was a low rate of adverse events
* Previous safety studies
  * 1x, 3x, 5x of 24 mg/kg dose at 12 hour intervals for 30 days
  * All adverse events were mild and self-limiting
  * Loose feces were most common and none were considered watery diarrhea
Bioequivalency Study
Oral suspension versus Oral paste

* Plasma concentrations
  * SDZ $C_{max}$ 176% of the paste
  * TMP $C_{max}$ 127% of the paste

* Lower dose
  * 24mg/kg Oral Suspension
  * 30mg/kg Oral Paste

* EQUISUL-SDT
  * Administered at 20% less drug than licensed paste product
  * Better absorption
    * Less exposure of intestinal flora to the antimicrobials