Comparison of Various Antibiotic Treatments for Cows Diagnosed with Toxic Puerperal Metritis

BILLY I. SMITH,* G. ARTHUR DONOVAN,‡ CARLOS RISCO,† RAMON LITTELL,‡ COLIN YOUNG,§ LARRY H. STANKER,§ and JESSIE ELLIOTT‡

*Aurora Dairy Corporation, 3830 SW 38th Street, Gainesville, FL 32608
‡Department of Large Animal Clinical Sciences, University of Florida, Gainesville 32610
†Institute of Food and Agricultural Sciences Department of Statistics, University of Florida, Gainesville 32610
§USDA-ARS, Food Animal Protection Research Laboratory, College Station, TX 77845

Received September 15, 1997. Accepted February 17, 1998.

ABSTRACT

Holstein cows (n = 51) that had been diagnosed with toxic puerperal metritis were used to determine the treatment efficacy of various antibiotics. On the day of diagnosis, cows affected with toxic puerperal metritis were assigned randomly to three treatment groups. Cows in groups 1 and 2 received 22,000 IU/kg of procaine penicillin G i.m. for 5 d. In addition, cows in group 2 received an intrauterine infusion of 6 g of oxytetracycline on d 1, 3, and 5. Cows in group 3 received 2.2 mg/kg of cefotiofur sodium i.m. for 5 d. Dependent variables used to determine antibiotic efficacy included milk yield on d 1 through 12, rectal temperature on d 1 through 5, and serum haptoglobin concentration on d 1, 3, and 5. No difference was observed among groups for milk yield on d 1 and 12 or for temperature on d 1 and 5. Serum haptoglobin was elevated to >10 mg/dl for cows in all groups; however, no difference was observed among groups on d 1 and 5. Because all groups showed a favorable response, this study suggests that there is no difference in treatment efficacy among antibiotics used to treat cows affected with toxic puerperal metritis. (Key words: toxic puerperal metritis, cow, antibiotic, haptoglobin)

Abbreviation key: DWS = detergent wash solution, RFM = retained fetal membranes, SHC = serum haptoglobin concentration, TPM = toxic puerperal metritis.

INTRODUCTION

Postpartum metritis is a common reproductive disease of dairy cows that occurs during the early postpartum period. Incidence can range from 3 to 36% (3, 23, 27). The degree of pathogenicity associated with uterine infections varies considerably and is dependent on a number of factors. In most cases, simple metritis is resolved by the defense mechanisms of the cow (10, 12, 15). However, the failure of these defense mechanisms might result in a toxic condition, toxic puerperal metritis (TPM), which is characterized by fever, anorexia, decreased milk yield, and a fetid, watery uterine discharge (19, 22). Systemic antibiotic treatment of TPM is crucial.

The determination of the exact microbial cause of TPM is difficult because of contamination by both pathogenic and nonpathogenic organisms and the fastidious nature of many of the isolates (28). However, many researchers (2, 19) support the theory that Gram-negative and Gram-positive anaerobes contribute to the development of TPM.

Compounds from numerous antimicrobial families can be used individually or in combination to treat TPM. These compounds include the \(\beta\)-lactams, tetracyclines, and sulfonamides. The recommended treatment for TPM is sytemic penicillin in conjunction with intrauterine oxytetracycline (18, 19, 29). However, the use of these antibiotics in dairy cows can result in milk residues that affect food safety. Cefotiofur sodium has broad-spectrum activity against many of the pathogens known to cause TPM and has no milk withdrawal time when used at the recommended dose. Currently, it is not known whether this drug is effective in treating dairy cows with TPM.

Recent research (1, 7, 13, 31) has focused on the use of haptoglobin as an indicator of the severity of disease. Haptoglobin is an acute phase reactant that is produced in response to a variety of inflammatory conditions (9). Cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor, are released in response to tissue damage and induce the liver to
produce acute phase proteins (1). Very little, if any, haptoglobin is present in the plasma of healthy cows (4). Our objectives were 1) to determine the clinical efficacy of various antibiotics in the treatment of TPM and 2) to determine serum haptoglobin concentration (SHC) in cows affected with TPM.

MATERIALS AND METHODS

Study Population

From April 1993 to November 1995, 51 Holstein cows diagnosed with TPM were enrolled in this study. Cows were from a 3500-cow dairy farm located in north central Florida. Study cows were managed and housed in a hospital facility on the farm, separate from the milking herd, and were fed a TMR that was formulated to meet or exceed NRC (17) requirements. In addition, Coastal bermudagrass hay was available for ad libitum intake.

Periparturient Cow Management

Cows within 3 wk of calving were maintained on pasture and were monitored for signs of calving every 2 h by farm employees who were trained to assist with parturition. Information was recorded for all births and included time of calving, size, number and health status of calves per birth, and difficulty of calving. Difficulty of calving was scored from 1 (cow needed no assistance) to 5 (cow needed a cesarean section). After calving, cows were examined per rectum to assess the status of the uterus, heart girth measurements were made to determine approximate weight, and a body condition score was assigned. Cows diagnosed with postpartum health abnormalities were assigned health codes, and medical treatment was administered if needed. Cows with retained fetal membranes (RFM) at the time of processing were recorded as RFM suspects and were reevaluated in 24 h. If at 24 h the cows had not expelled the fetal membranes, they were recorded as having RFM. These cows were given an intrauterine infusion of 3 g of tetracycline hydrochloride (Panmycin®, The Upjohn Co., Kalamazoo, MI), which is an extra-label use of this drug.

Cows were milked twice daily at the hospital barn; morning milk weights were recorded for all cows. Cows in the hospital barn were kept in dirt lots. Shade was provided by permanent structures with a wood frame, a metal roof, and a portable nylon mesh shade.

Definition of Disease

The investigator and trained farm personnel identified cows that were eligible for this study. All cows identified by farm personnel were reevaluated by the investigator to ensure accuracy of disease identification. All cows that were eligible for the study were required to meet the following two criteria that were used to diagnose TPM: 1) cows must have been febrile (i.e., morning rectal temperature >39.2°C) and 2) cows must have had a flaccid, nonretractable uterus that was located in the abdomen, a cervical diameter >75 mm, and a watery, fetid vulvar discharge. Other criteria used to diagnose TPM included depressed milk yield (<7.4 kg at the morning milking) for a cow that was 3 to 20 d postpartum.

Study Design

Once diagnosed, cows were randomly assigned to one of three treatment groups, each representing a different antibiotic treatment. Treatment groups 1 and 2 received once daily an i.m. injection of procaine penicillin G (Pen-Aqueous®, RX Veterinary Products, Porterville, CA) at a dose of 22,000 U/kg of BW for 5 consecutive d. Procaine penicillin G was administered at a dose that was nearly four times the dose suggested on the label by the manufacturer (6600 U/kg). In addition to the penicillin treatment, cows in group 2 received an intrauterine infusion of 6 g of oxytetracycline (Oxyject®, Agri Laboratories Ltd., St. Joseph, MO) diluted in 75 ml of sterile water (Agri Laboratorie Ltd.) on d 1, 3, and 5. The method of administration was not one of the recommended routes of administration according to the manufacturer and thus is an off-label use of the drug. Cows in group 3 received once daily an i.m. injection of ceftiofur sodium (Naxcel®, The Upjohn Co.) at a dose of 2.2 mg/kg of BW for 5 consecutive d. Because no dose has been established for bovine uterine infections, ceftiofur sodium was administered at the dose recommended by the manufacturer on the label. All i.m. injections were administered in the neck or low semimembranosus or semitendinosus muscles. No untreated control group was included in this study. Cows enrolled in the study presented signs of a potentially life-threatening systemic illness, and the decision was made to treat all affected cows.

Data Collection and Handling

Milk yield was measured at the morning milking and was recorded from d 1 through 12 using a glass jar located at each milking unit. Milk weights on d 1 were recorded prior to the diagnosis and treatment of the cows.
Rectal temperatures of each cow were taken and recorded on d 1 through 5 using a glass thermometer. Temperatures were taken between 0500 and 0800 h after the morning milking.

Blood was collected via coccygeal venipuncture using evacuated tubes (Vacutainer®; Fischer Scientific, Itasca, IL). Blood collected on d 1 was taken before treatment was administered. Blood was allowed to coagulate and then was refrigerated at 4.4°C. Clots were removed from the serum within 24 h of collection, and serum was collected after centrifugation at 4500 \( \times \) g. Serum was then stored frozen at \(-6.7\)°C until time of analysis.

**Haptoglobin Analysis**

The SHC was measured using enzyme immunoassay on thawed serum collected on d 1, 3, and 5 (30). The methodology used to measure SHC has been described previously by Young et al. (31). A standard curve was run concurrently with each plate analysis for SHC. Bovine hemoglobin (20 \( \mu \)g in 200 \( \mu \)l of 50-mm carbonate buffer; pH 9.6) was used to coat microtiteration plates (NUNC; PGC Scientific, Fredrick, MD), which were then allowed to stand overnight at 4°C. Prior to use, 3% NDM in PBS solution (pH 9.0) was used to block untreated sites on the plates, and the plates were then incubated for 60 min at 37°C. After the blocking solution was removed, the plates were washed three times in PBS solution (pH 9.0). Dilutions of bovine test sera [in 1% NDM in PBS solution (pH 7.0)] containing haptoglobin were then added to plates that had been coated with hemoglobin and incubated for 1 h at 37°C. After incubation, the plates were drained and washed five times in detergent wash solution (DWS) [0.05% polysorbate 20 in deionized water detergent (polyoxyethylene-sorbitan monolaurate)] followed by five washes in deionized water. To each well were added 100 \( \mu \)l of a 1:1000 (vol/vol) dilution of purified anti-haptoglobin monoclonal antibody derived from mice [in 1% NDM in PBS solution (pH 7.0)]; wells were then incubated for 1 h at 37°C. After incubation, the plates were drained, washed five times with DWS, and washed five times in deionized water. Then, 100 \( \mu \)l of goat anti-mouse IgG whole-molecule peroxidase conjugate (Sigma Chemical Co., St. Louis, MO) diluted 1:500 (vol/vol) [in 1% NDM in PBS solution (pH 7.0)] were added to each well and incubated for 1 h at 37°C. After incubation, the plates were drained, washed five times with DWS, and washed five times in deionized water. Finally, 100 \( \mu \)l of substrate (K-blue; Neogen Corp., Lexington, KY) were added to each well, plates were incubated for 20 min at room temperature (20 to 22°C), and the optical density at 650 nm was recorded.

**Statistical Analyses**

Statistical analyses were performed by mixed model methodology using PROC MIXED of SAS/STAT (24). Dependent variables in the analyses included milk yield, rectal temperature, and SHC. All data were analyzed beginning on d 2. Initial milk weight as a covariable was considered but was not used in the final analyses.

For analysis of milk yield, two initial milk weight response outliers, one from a cow in group 1 and one from a cow in group 2, were eliminated from the final analyses. The discrete independent variables in all statistical models were treatment group and cow. Day was treated as a continuous independent variable using second degree polynomials for each group.

For analysis of rectal temperature and SHC, the

---

**Table 1. Final distribution of cows by treatment group and statistical description of parity and DIM for treatment groups.**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>no.</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Parity</td>
<td>17</td>
<td>1.71</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>DIM</td>
<td>17</td>
<td>6.53</td>
<td>7</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>2 Parity</td>
<td>17</td>
<td>1.88</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>DIM</td>
<td>17</td>
<td>6.06</td>
<td>5</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>3 Parity</td>
<td>17</td>
<td>1.53</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>DIM</td>
<td>17</td>
<td>6.41</td>
<td>6</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

1Treatment groups: 1 = i.m. injection of procaine penicillin G, 2 = i.m. injection of procaine penicillin G plus intrauterine injection of oxytetracycline, and 3 = i.m. injection of cefiotaxim sodium.
discrete independent variables in all statistical models were treatment group, cow, and day. Because of the relatively small number of days for which rectal temperature and SHC were measured and because of the erratic trends for rectal temperature over days, day was treated as a discrete variable.

Cow nested within group was considered a random effect, which was equivalent to assuming a compound symmetric covariance structure. Because of the extreme heterogeneous variation among values, haptoglobin data were log10-transformed.

RESULTS

A total of 51 cows was used in this study. Thirty of 51 cows (59%) in this project were primiparous. Cows that did not meet the diagnostic criteria on d 1 were removed as were any cows that suffered from diseases other than TPM. All cows that received the antipyretic agent flunixin meglumine (Banamine®, Schering-Plough Animal Health Corp., Kenilworth, NJ) were removed because rectal temperature was an important variable. Within 3 to 20 DIM, 50 of 51 cows met the established criteria that were used to diagnose TPM. Table 1 shows the final distribution of cows by treatment group and the statistical description of lactation and DIM for each treatment group. A similar mean lactation number and number of DIM for cows in all three treatment groups indicated a successful randomization of the study.

A seasonal calving pattern was used on the farm where this project was conducted; the majority of calvings occurred from August to December of each year. In this project, 34 of 51 cows (67%) calved between June and September, which are traditionally the hottest months in Florida.

The majority of cows (28 of 51; 55%) in this project had RFM. A total of 50 cows received calving codes. Most cows (40 of 50; 80%) had a score of ≤2, but a few (10 of 50; 20%) had a score of ≥3. No cow needed a cesarean section.

Two measurements of milk yield were eliminated because of inconsistencies. Milk yields on d 1 for 1 cow in group 1 (15.5 kg) and that for 1 cow in group 2 (25.9 kg) were eliminated from the data used in the analyses. The initial milk yield for the cow in group 2 was much higher than the mean milk yield of cows in early lactation. This suspiciously high measurement could have been the result of an error in reading the milk weight. The cow in group 1 was eliminated because her initial milk weight was high and not comparable with subsequent milk weights, which could have also been due to an error in measuring or recording data.

The change in milk yield from d 2 through 12 should reflect the response of the cow to treatment. In each treatment group, milk yield curves followed a similar pattern during the trial (Figure 1). Comparisons of milk yields among groups on d 1 and 12 of the trial showed no difference (P > 0.05) among treatment groups. However, cows in group 1 tended to have lower milk yields than did cows in groups 2 (P = 0.19) and 3 (P = 0.10) on d 1. On d 1, cows in group 1 had a lower least squares mean estimate of milk yield than did cows in groups 2 and 3. The final estimates of milk yield on d 12 were very similar for all groups.

Day and treatment group affected (P < 0.05) milk yield. Cows in all groups showed a rapid increase in milk yield over the first 8 d of the study. This increase gradually reached a plateau from d 8 to 12. No interaction of day and treatment group was detected. Over the 12 d of measurement, the milk yield of cows in group 1 was different (P < 0.05) from the milk yields of cows in groups 2 and 3.
of cows in groups 2 and 3. No difference \((P > 0.05)\) in milk yield was detected between groups 2 and 3.

Analysis of data showed that initial temperatures for all three groups were comparable as were decreased temperatures on d 5 (Figure 2). Comparisons of rectal temperatures among treatment groups on d 1 and 5 showed no differences \((P > 0.05)\). Cows in group 3 started at a higher least squares mean estimate \((P > 0.05)\) for temperature than did cows in groups 1 and 2. However, the final estimates on d 5 were very similar for all groups. Day affected temperature \((P < 0.05)\), but treatment group did not \((P > 0.05)\). No interaction of day and treatment group was detected.

Table 2 shows the means, standard deviations, minimums, and maximums for SHC values. In each treatment group, the SHC curves followed a similar pattern (Figure 3). Day affected SHC \((P < 0.05)\), but treatment group had no effect \((P > 0.05)\). No interaction of day and treatment group was detected. Cows in groups 1 and 2 showed a steady decrease in SHC from d 1 through 5. Values on d 1 and 3 for cows in group 3 were very similar but decreased from d 3 to 5.

A comparison of the least squares mean estimates for SHC \((\log_{10} \text{ milligrams per deciliter})\) among the three treatment groups during the first 5 d of the study was made. Although treatment groups were not different \((P \geq 0.05)\) on d 3 and 5, cows in group 1 tended to have lower SHC than did cows in group 3 \((P = 0.05)\) on d 5. Cows in groups 2 and 3 started at a similar mean SHC on d 1, and this value was higher than that of cows in group 1. By d 5, SHC in all cows decreased to a similar concentration, which was within the normal physiologic limit \(< 10 \text{ mg/dl}\). Twelve of 51 cows (group 1 = 3, group 2 = 4, and group 3 = 5) had SHC < 10 mg/dl on d 1. Two of 51 cows had no detectable concentrations of haptoglobin on d 1.

**DISCUSSION**

Toxic puerperal metritis affects dairy cows of different parities. The speculation that primiparous heifers are more likely to develop TPM may stem from the higher occurrence of dystocia in this group of dairy cows. Literature reports concerning the association of parity and metritis offer mixed opinions. Markusfeld (14) reported a higher risk of metritis in heifers. However, Pugh et al. (21) found no association between cases of puerperal metritis and parity of the cow. In this study, the higher number of primiparous heifers affected with TPM was likely due

---

**Figure 2.** Least squares means for morning rectal temperatures of cows affected with toxic puerperal metritis and treated with different antibiotics. Legend: cows treated with i.m. injection of procaine penicillin G (●) \((\text{SEM} = 0.10)\), cows treated with i.m. injection of procaine penicillin G and intrauterine injection of oxytetacycline (▲) \((\text{SEM} = 0.10)\), and cows treated with i.m. injection of ceftiofur sodium (■) \((\text{SEM} = 0.10)\).
The occurrence of TPM in this study was during the early postpartum period, which agrees with the published research on the etiology of the disease. Gilbert and Schwark (11) described the disease as occurring within the 1st wk postpartum. In addition, Olson et al. (19) stated that life-threatening infections occur almost exclusively during the puerperal period, which they defined as the period from the time of calving until 7 to 14 d postpartum. The development of TPM within 72 h of calving is likely due to infection by a highly pathogenic organism, to poor resistance of the cow, or to the occurrence of multiple predisposing factors in these cows.

The uterus of the postpartum cow has been cultured extensively, and a variety of bacteria has been isolated. Metritis caused by bacteria such as Clostridium spp. is often a toxic, gangrenous metritis. Also, the presence of Arcanobacterium pyogenes and Gram-negative anaerobic organisms such as Fusobacterium necrophorum and Bacteroides spp. enhance the growth and pathogenicity of one another, which may result in a severe life-threatening uterine infection (20).

The defense mechanisms of the cow against a uterine infection include anatomical barriers, cervical mucus, uterine fluids, antibodies, and phagocytic cells. The efficacy of these mechanisms in the prevention of uterine infections is dependent on the stage and predominant hormone of the reproductive cycle. Also, the failure of these defense mechanisms may be related to immunosuppression, intrauterine placement of chemical compounds, and periparturient disorders.

Normally, a postpartum cow has a steady increase in milk yield after parturition. The increase continues until a peak is reached at about 60 d postpartum. Milk yield is affected by disease. Cows suffering from a severe life-threatening condition tend to yield less milk. Data are limited on the actual amount of milk lost because of TPM. However, the mean milk yields on d 1 for cows in all treatment groups in this study were comparable with yields obtained from cows suffering from TPM as reported by Deluyker et al. (8). The increase in milk yield after the initiation of treatment as well as the similar milk yield records at the end of the trial indicated a successful treatment response for all three groups.

By trial design, the mean temperature on d 1 for cows in all treatment groups was above the normal body temperature (39.2°C) and was consistent with clinical signs observed in cows (11, 21). Cows in all groups had similar rectal temperatures on d 5, indicating an equally successful treatment response for all three groups. The elevated temperatures observed on d 1 support the diagnosis of TPM. These values were similar to the rectal temperatures reported by Pugh et al. (21) for cows with TPM. The temperature of cows in all groups decreased steadily after treatment was initiated; the decrease was marked from d 1 to 3. Antimicrobial agents were the only medical treatment given to the cows in this study.

Cows in all three groups had elevated SHC at the beginning of the trial. The results in this study agree with the findings of Skinner et al. (25). Those investigators found that SHC in cows suffering from TPM were elevated to >10 mg/dl.

The low SHC observed in cows diagnosed with TPM could have been the result of a number of different factors. One possible explanation for this finding to the higher number of heifers calving during the time the study was conducted.

Figure 3. Least squares means of serum haptoglobin concentrations for cows affected with toxic puerperal metritis and treated with different antibiotics. Legend: cows treated with i.m. injection of procaine penicillin G (●) (SEM = 1.85), cows treated with i.m. injection of procaine penicillin G and intrauterine injection of oxytetracycline (▲) (SEM = 2.91), and cows treated with i.m. injection of cefiofur sodium (■) (SEM = 3.73).
might be related to the handling, storage, and testing of the serum. However, in this study, all samples were managed in the same manner. Another explanation is related to the individual variation of the cows. In cows with low SHC, TPM might have been diagnosed early in the disease process. Therefore, blood was collected before a rise in SHC could have been observed. In addition, the virulence of the specific agent that caused the disease could have influenced the SHC measured in these cows. Cows with TPM caused by a highly pathogenic organism may possibly have higher SHC.

Haptoglobin may be helpful in the determination of the severity of the disease, although severity per se was not measured in this study. The initial least squares mean value for SHC for each group was >10 mg/dl, which indicates an acute infectious process with a high degree of severity and supports the correct diagnosis of TPM. These results are in agreement with other researchers (4, 6, 16, 25, 26) who studied serum haptoglobin of cows suffering from diseases causing inflammation.

The decrease in SHC observed after the initiation of treatment was most likely due to the positive effects of the antimicrobial treatment administered during the first 5 d of the study. Effective treatment results in a reduction of intraterine bacteria as well as a concurrent decrease in white blood cells and inflammation, which could explain the lower SHC observed after antimicrobial treatment was initiated.

The association between SHC and clinical disease is difficult to understand. Apparently, SHC may not be an accurate predictor of the occurrence or severity of disease. However, this study and several others have shown that SHC is elevated during inflammatory diseases such as TPM. The SHC alone may provide limited information but may be useful when used in conjunction with other diagnostic procedures such as rectal palpation, fibrinogen analysis, or cytokine (interleukin-6) analysis (1, 31). Therefore, further research is needed in this area to understand fully the importance of SHC and how it can be used in the analysis of bovine inflammatory diseases.

The increase in milk yield, decrease in rectal temperature, and decrease in SHC after initiation of treatment as well as the similar values observed at the end of the study indicated a successful treatment response for all three groups. The pharmacokinetic principles of ceftiofur sodium might explain the efficacy of this product on uterine infections. Ceftriaxone sodium is a water-soluble drug that partitions into extracellular fluids. Because of this characteristic, ceftiofur sodium should partition into the extracellular fluids of the uterus. Clarke et al. (5) studied the localization of ceftiofur sodium at infected areas. That study revealed that both HPLC and microbiologically active metabolites were higher in infected sites than in uninfected sites. Also, metabolites bound to protein serve as reservoirs for the microbiologically active drug at the site of infection. This reservoir of ceftiofur sodium should enable the drug to reach efficacious concentrations in the uterine fluid for an extended period. These basic pharmacokinetic principles of ceftiofur sodium and the findings of this research trial illustrate the use of this drug in the treatment of TPM.

**CONCLUSIONS**

Cows that were diagnosed with TPM responded positively to three different antimicrobial treatment regimens. No differences among treatment groups were detected when rectal temperature and SHC were measured. A difference was found among treatment groups when milk yield was measured; however, all groups showed an overall positive response in milk yield.

This study also showed that cows diagnosed with TPM had elevated SHC, and SHC declined markedly following antimicrobial treatment. Further work is needed to determine the value of SHC measurement in cows with infectious diseases. Results from this study indicate that ceftiofur sodium is an alternative choice in the treatment of TPM.

**REFERENCES**