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A study to examine the relationship between metritis severity and depletion of oxytetracycline in plasma and milk after intrauterine infusion

P. J. Gorden,*[1] J. A. Ydstie,* M. D. Kleinhenz,* L. W. Wulf,*† R. Gehring,‡ C. J. Lee,*†[2] C. Wang,* and J. F. Coetzee*†[1]
*Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, and
†Pharmacology Analytical Support Team, Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames 50011
‡Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan 66502

ABSTRACT

Metritis is a frequent problem in postpartum dairy cows. Intrauterine therapy with the antimicrobial oxytetracycline (OTC) is often used, although this therapy has not been shown to be superior to systemic therapy. The objectives of this study were to (1) determine the plasma and milk concentrations of OTC following intrauterine infusion in postpartum dairy cows with varying degrees of metritis severity; (2) determine the depletion time of OTC in an attempt to provide veterinarians withdrawal guidelines, should they use this therapy; and (3) correlate metritis severity scores with OTC concentrations in plasma and milk. Our hypothesis was that cows with more severe metritis would have higher OTC concentrations in milk following intrauterine therapy. Thirty-two cows were selected to participate in the study after farm personnel had determined that they had metritis based on evaluation of vaginal discharge between 4 and 14 DIM, in accordance with the farm's treatment protocols. Metritis scores (1–4) were assigned based on a published scheme: 1 represented yellow-to-orange thick discharge or translucent mucus with no fetid smell; 2 represented blood-tinged vaginal mucus, slightly watery, with little or no fetid smell; 3 represented red to red/brown watery discharge with moderate fetid smell; and 4 represented red to red/brown watery discharge containing pieces of placenta and an intense fetid smell. Trial cows received a single treatment of 4 g of OTC (approximately 6.7 mg/kg) via intrauterine infusion. Blood samples were collected over 96 h, and milk samples were collected before intrauterine therapy and 3 times a day for 4 d following infusion. Following treatment, OTC rapidly diffused to plasma and subsequently to milk. Maximum OTC concentrations in plasma and milk occurred within the first 24 h following intrauterine infusion, and 25 of the 32 cows had detectable OTC concentrations in milk at 4 d after intrauterine infusion. Cows with clinical metritis (metritis severity scores of 3 or 4) at the initiation of treatment were significantly and positively correlated with higher milk OTC concentrations at the second [time (T)9 h; r = 0.43], fourth (T25 h; r = 0.42), and fifth milking following treatment (T33 h; r = 0.38) compared with cows with normal vaginal discharge. We also observed a positive correlation between initial metritis score and milk maximum concentration (r = 0.36) and milk area under the concentration curve (r = 0.36). Given that intrauterine administration of OTC is an extra-label therapy, dairy producers should consult with their veterinarian to ensure that milk is being tested at or below the established tolerance for OTC. This will ensure that violative drug residues do not enter the human food supply.

Key words: bovine metritis, oxytetracycline, pharmacokinetics, drug residues

INTRODUCTION

Bacterial contamination of the uterus occurs in 90% or more of dairy cows during the first week after calving (Sheldon and Dobson, 2004). Interestingly, bacterial contamination of the postpartum uterus has been described as dynamic, with contamination, clearance, and recontamination occurring throughout the postpartum period (Griffin et al., 1974). The dynamic status of the intrauterine environment results in much lower rates of clinical metritis and endometritis than would be expected given the high levels of bacterial infection in the first week postpartum. The prevalence of metritis in postpartum cows can range from 18 to 33%; however, accurate estimates are difficult to obtain due to inconsistencies in clinical disease definitions among modern dairy farms (Markusfeld, 1987; Dril-

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[1]Corresponding authors: pgorden@iastate.edu and hcoetzee@iastate.edu
[2]Current address: Division of Food and Environmental Sciences, Wonkwang University, Iksan, Jeonbuk, South Korea.

lich et al., 2001; Benzaquen et al., 2007). *Trueperella pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum*, and *Prevotella melaninogenicus* are the bacteria most commonly isolated from cases of clinical metritis. These bacteria are distributed throughout all layers of the uterus during cases of clinical metritis, making parenteral therapy a more effective choice for treatment (Sheldon and Dobson, 2004).

Current treatment practices on US dairy farms for common diseases, including metritis, are not well documented in the literature. Recently, our research group conducted a survey of 83 Midwest dairy farms to document treatment practices on these farms. The survey found that 58% of farms practiced at least some intrauterine therapy for metritis, and just over half of these farms used oxytetracycline (**OTC**) in their infusions (P. J. Gorden, unpublished data). Although intrauterine antimicrobial therapy is used on dairy farms in some parts of the United States, the results of studies designed to assess improvements in uterine health or financial outcomes have not shown benefits from the use of intrauterine therapy alone or in combination with systemic treatments (Smith et al., 1998; Drillich et al., 2001). However, repeated intrauterine therapy with chlortetracycline has been shown to be superior to no treatment (Goshen and Shpigel, 2006). As well, systemic absorption of antibiotics following intrauterine infusion has been reported. This can result in costly milk discard and is a risk for antibiotic residue in bulk tank milk intended for human consumption (Dinsmore et al., 1996). Intrauterine antibiotic administration in cattle in the United States is considered an extra-label therapy and is governed by the Animal Medicinal Drug Use Clarification Act (21CFR530; US FDA, 1996). Under these regulations, the prescribing veterinarian must determine if extra-label therapy is justified and allowable. The prescribing veterinarian is also responsible for specification of a withdrawal time for milk and meat that ensures that products from treated cows are below the tolerance levels for antimicrobial residues, as well as properly documenting extra-label therapy. Previous trials have also reported considerable variation in the depletion profile of drugs from milk following intrauterine infusion (Kaneene et al., 1986; Anderson et al., 1995; Dinsmore et al., 1996). Such variation may be associated with the severity of metritis and resulting inflammation, which could have an effect on drug absorption.

Designing a system to assess the severity of metritis in postpartum cows is a subject of debate in the literature, and no gold standard has been established (Wenz et al., 2011; Sannmann et al., 2012; de Boer et al., 2014). Some suggest that the severity of metritis should be graded based on abnormal character and odor of the vaginal discharge, along with a rectal temperature >39.5°C (Sheldon et al., 2009). Others suggest that a large percentage of animals with severe metritis will be missed when including rectal temperature >39.0°C (Palenik et al., 2009) or >39.3°C (Benzaquen et al., 2007) as a diagnostic criterion. In cows with endometritis, the clinical evaluation of the character and odor of vaginal discharge has been associated with pathogenic bacterial load (Williams et al., 2005). Because farm personnel were using transrectal uterine massage to express uterine contents and grade the clinical severity of metritis without using rectal temperature, we modified previously described uterine severity scoring systems (Benzaquen et al., 2007; Sheldon et al., 2009) to assess metritis severity in trial cows.

Before the 2015 Pasteurized Milk Ordinance (**PMO**), scheduled testing of drugs other than β-lactams was not required for milk marketed as grade A in the United States (US FDA, 2013). However, although it is not required, dairy processors can still test tanker milk for the presence of tetracycline-class drugs if they wish. At the 2015 National Conference on Interstate Milk Shipments (NCIMS), proposal 211 was passed requiring the development of a more comprehensive milk residue-testing program under Appendix N of the PMO, starting with the 2015 version (US FDA, 2015a). As a result, regular screening for drugs such as OTC could be undertaken for regulatory purposes in the near future.

The objective of this study was to use highly sensitive laboratory technology to determine the plasma and milk concentrations of OTC following intrauterine infusion in postpartum dairy cows. We also hoped to characterize OTC depletion from plasma and milk to better determine milk withdrawal times and correlate OTC concentrations throughout the study with metritis severity scores. We hypothesized that variability in distribution and elimination of OTC from the uterus to plasma and milk would be associated with the severity of metritis, as assessed by metritis scores. Specifically, we postulated that cows experiencing more severe metritis would have higher plasma and milk OTC concentrations than less severely affected cows.

MATERIALS AND METHODS

Animals and Eligibility Criteria

Cows were housed in a privately owned, commercial confinement dairy facility with sand-bedded free stalls and manger headlocks. Our protocol complied with the requirements of Iowa State University's Institutional Animal Care and Use Committee. The trial herd had a 305-d mature-equivalent milk production of 10,935 kg. During the pre- and postpartum periods, cows were