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Noninferiority trial comparing a first-generation cephalosporin with a third-generation cephalosporin in the treatment of nonsevere clinical mastitis in dairy cows

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ABSTRACT

The objective of this study was to evaluate the noninferiority of 2 intramammary treatments for nonsevere clinical mastitis. The 2 treatments were a first-generation cephalosporin (cephapirin sodium, 2 treatments 12 h apart) and a third-generation cephalosporin (ceftiofur hydrochloride, treatments once a day for 5 d). A total of 296 cases on 7 farms met the enrollment criteria for the study. *Streptococcus dysgalactiae* was the most common bacterial species identified in milk samples from cows with mild to moderate clinical mastitis, followed by *Escherichia coli,* other esculin-positive cocci, *Streptococcus uberis*, and *Klebsiella* spp. Treatment was randomly allocated as either cephapirin sodium or ceftiofur hydrochloride via intramammary infusion according to label standards. Bacteriological cure was defined based on 2 posttreatment milk samples taken at 10 and 17 d after enrollment. Noninferiority of cephapirin relative to ceftiofur was shown for bacteriological cure of gram-positive cases and for clinical cure of all cases. Ceftiofur showed a significantly higher bacteriological cure in gram-negative cases. Treatments showed no significant difference in bacteriological cure of all cases and in time to exit from the study, where the absence of a difference does not imply noninferiority. Based on the findings from this study, farm-specific treatment protocols that differ for gram-positive and gram-negative cased may be developed.
Key words: mastitis, cephapirin, clinical trial, noninferiority

INTRODUCTION

Clinical mastitis (**CM**) is the most important disease of dairy cattle (Barkema et al., 1998; Gröhn et al., 2004). Farmers try to combat the disease and minimize the losses due to the disease by treating animals (Roberson, 2012). Several treatment options are available to treat CM; however, relatively few studies have compared the efficacy of available treatments under comparable circumstances (Waage, 1997; Deluyker et al., 1999; Taponen et al., 2003; Roberson et al., 2004; McDougall et al., 2007, 2010; Bradley and Green, 2009), and few of these studies were designed as noninferiority studies. Most published randomized controlled trials have been designed to determine whether a treatment is superior to an untreated control. However, efficacy compared with a negative control is not the primary interest of the dairy producer. It can be argued that comparison with existing treatments that are on the market is of more value for day-to-day decision making (Roberson, 2012). Also, studies that aim to demonstrate equivalence or noninferiority of treatments should be used when there are ethical concerns about leaving animals untreated (Schukken and Deluyker, 1995; Piaggio et al., 2006; O'Connor et al., 2010). Equivalence or noninferiority is particularly valuable and valid when there are advantages such as reduced costs, reduced dosing frequency, or improved safety of one product compared with a reference product (Piaggio et al., 2006; Powers, 2008). In the United States, placebo or no treatment controlled trials are the norm for registration of products (US FDA-CVM, 1996), whereas in the European Union, a positive control trial is recommended for registration of products in their market area, unless a placebo or no treatment control can be justified (European Medicines Agency, 2009). Throughout the world, several intramammary products for the treatment of CM in dairy cattle contain cephalosporins. Four generations of cephalosporins have evolved, all of which contain the β-lactam sub-structure first found in penicillin. The mastitis treatment products on the US market fall in the first and third cephalosporin generations. First-generation cephalosporins generally have a gram-positive spectrum with limited gram-negative activity (Guérin-Faublée et al., 2003; Pfeifer et al., 2010), whereas third-generation products have a broader spectrum of activity (Hornish and Kotarski, 2002). For this reason, there is a specific interest in

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the efficacy of third-generation cephalosporins against gram-negative organisms (Erskine et al., 2002; Wenz et al., 2005; Schukken et al., 2011).

Although gram-negative organisms are an important cause of CM on well-managed dairies (Erskine et al., 1988; Barkema et al., 1998; Olde Riekerink et al., 2008), gram-positive organisms such as *Streptococcus uberis*, *Streptococcus dysgalactiae*, and CNS remain present in a high number of treated mastitis cases (Apparao et al., 2009). Further, CM with culture results of "no growth" may represent 30 to 40% of cases (Olde Riekerink et al., 2008; Lago et al., 2010). Therefore, third-generation cephalosporins may not necessarily have an easily identifiable advantage over first-generation cephalosporins on all farms.

Even more, concerns over the use third- and fourth-generation cephalosporins in food animals may eventually result in a more restrictive use of these antibiotics (Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use, 2009). Recently, the US Food and Drug Administration issued an order that prohibits the extra-label use of cephalosporin drugs (specifically excluding cephapirin) in cattle and other species (http://www.gpo.gov/fdsys/pkg/FR-2012-01-06/pdf/2012-35.pdf). Similar or more restrictive use of third- and fourth-generation cephalosporins is anticipated in European countries (Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use, 2009). Hence, despite the potential benefits of third-generation cephalosporins over first-generation cephalosporins (Schukken et al. 2011), first-generation cephalosporins may eventually be more easily accepted in the dairy industry.

In this study, we compared the treatment efficacy of a 1-d treatment (2 doses at a 12-h interval) with the test product containing a first-generation cephalosporin (cephapirin) to the reference treatment of 5-d once-a-day treatment of a third-generation cephalosporin (ceftiofur). The objective of the trial was to evaluate whether noninferiority of the test product against the reference product could be established.

MATERIALS AND METHODS

The study was conducted on commercial dairy farms in New York State between December 2010 and September 2011.

Study Design

The study design for this treatment comparison study was a noninferiority study. Proving equality of treatments is logically impossible, so a pre-stated margin of noninferiority ($\boldsymbol{\Delta}$) needs to be defined (Piaggio et al., 2006; O'Connor et al., 2010). In noninferiority studies, the null hypothesis is that one treatment is inferior to the reference product, and the alternative hypothesis is that the new treatment is not inferior by more than the predefined margin ($-\Delta$; Piaggio et al., 2006). Thus, rejecting the null hypothesis (H_0) results in acceptance of the alternative hypothesis (H_A) that the new product is noninferior to the reference product, more formally written as

$$H_0: [P_{\text{cure}}(\text{cephapirin}) - P_{\text{cure}}(\text{ceftiofur})] \leq -\Delta$$

$$H_A: [P_{\text{cure}}(\text{cephapirin}) - P_{\text{cure}}(\text{ceftiofur})] > -\Delta,$$

where P_{cure} is the probability of cure and Δ is the noninferiority margin. In Figure 1, the principle of a noninferiority study is shown and the possible decisions based on hypothetical study outcomes are explained (adapted from Piaggio et al., 2006).

Sample Size Determination

The required sample size was calculated using the confidence interval (**CI**) approach, considering where the CI for the treatment effect lies with respect to both the margin of noninferiority, Δ, and a null effect (treatments are assumed a priori to show equal cure risks). Selection of Δ is often based on results of negative (or placebo) controlled studies, on the basis that Δ will be no more than half the effect expected from a superiority study (Piaggio et al., 2006; Powers, 2008). Sample size was calculated assuming that the ceftiofur cure risk was approximately 70% (Schukken et al., 2011) and a statistical significance of 5% and power of 80% were chosen. For this study, we defined the acceptable difference in cure, the margin of noninferiority (Δ), as 0.15. The choice of the noninferiority margin was based on a recent study in which ceftiofur-treated cows with mild or moderate CM caused by gram-negative organisms showed a 35% higher bacteriological cure compared with untreated controls (Schukken et al., 2011). The noninferiority margin of 0.15 is less than half this effect estimate from a superiority study. The same noninferiority margin has been used and suggested in other CM studies (Schukken and Deluyker, 1995; Deluyker et al., 1999). This choice of noninferiority margin resulted in an estimated sample size per group of 110 cases. The total study size is then twice this number at approximately 220 cases. Sample size calculations were performed with the use of StudySize 2.0.4 (Creostat HB, www.creostat.com). To allow for animals that were culture negative at first detection of CM, the objective was to enroll 300 cows in the study. We thereby