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Evaluation of the efficacy of intramuscular versus intramammary treatment of subclinical *Streptococcus agalactiae* mastitis in dairy cows in Colombia

J. Reyes,*[1] M. Chaffer,* J. Sanchez,* G. Torres,† D. Macias,‡ M. Jaramillo,‡ P. C. Duque,§ A. Ceballos,§ and G. P. Keefe*

*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, C1A 4P3 Canada
†Tropical Medicine Colombian Institute, CES University, CRA. 43A No. 52 sur-99 Medellín, Antioquia, Colombia
‡Cooperativa COLANTA, CRA 64C, 72-160 Medellín, Colombia
§Department of Agricultural and Livestock Sciences, Universidad de Caldas, Calle 65 #26-10, Manizales, Caldas, Colombia

ABSTRACT

A randomized controlled trial was performed in 17 Colombian dairy herds to determine the cure risk among cows subclinically infected with *Streptococcus agalactiae* exposed to 2 antibiotic therapies. Composite milk samples were collected before milking at the onset of the trial (pretreatment) and 2 subsequent times over a period of approximately 63 d. The intramammary application (IMM) of ampicillin-cloxacillin was compared with the intramuscular application (IM) of penethamate hydriodide, and cure risks after an initial and retreatment application were assessed. Cure risk after the initial treatment was higher (82.4%) for the IMM treatment than for IM therapy (65.8%). However, no difference was observed in the cure risk of refractory cases after retreatment (IMM = 52.6% vs. IM = 51.2%). The cumulative cure risk (both initial and retreatment) was 90.4 and 82.9% for the IMM and IM products, respectively. A 2-level random effects logistic model that controlled for pretreatment cow-level somatic cell count, indicated that IM treatment (odds ratio = 0.37) had a lower cure risk than IMM and a tendency for a lower cure risk with increasing baseline somatic cell count. Our findings suggest that both products and administration routes can reduce the prevalence of *S. agalactiae* in affected herds, but the IMM product had a better efficacy in curing the infection. In addition to the treatment protocol, the cow somatic cell count should be considered when making management decisions for cows infected with *S. agalactiae.*

Key words: somatic cell count, *Streptococcus agalactiae*, subclinical mastitis, antibiotic

INTRODUCTION

Bovine mastitis continues to be the most economically important disease in dairy cattle (Gröhn et al., 2004) and is caused by a broad spectrum of infectious agents. *Streptococcus agalactiae* is considered to be a major contagious pathogen for bovine mastitis, and the primary reservoir of the pathogen and source of infection for healthy animals is the udder of infected herdmates (Keefe, 1997).

The prevalence of *S. agalactiae* has been reduced in North American and European countries with long-standing extension programs (Keefe, 2012). However, in the Scandinavian countries, particularly Denmark a reemergence of *S. agalactiae* has been documented (Zadoks et al., 2011; Katholm et al., 2012). Without systematic surveillance it is uncertain if this is also the case in other European countries or North America. By contrast in South America, *S. agalactiae* has consistently remained an important pathogen, with a herd-level prevalence of 60% in Brazil and 42% in Colombia, and a cow-level prevalence of 11% in Uruguay (Gianneechini et al., 2002; Duarte et al., 2004; Keefe et al., 2011). In a Colombian study, among quarters with elevated California mastitis test, 34.7% had *S. agalactiae* (Ramírez et al., 2014).

A recent study in Colombia showed that the presence of *S. agalactiae* in a herd has a significant effect on milk quality. That study reported that positive herds had a 70% higher bulk tank milk somatic cell count (**BTSCC**) than negative herds. Moreover, the total bacteria burden in the positive tanks was almost twice as high as in the negative tanks (Keefe et al., 2011).

The control of this pathogen remains important from a global health perspective. The presence of this pathogen in the cows and bulk tank suggests significant control problems in the herd (Edmondson, 2011) and the need to improve within-herd biosecurity (Keefe, 2012). Eradication of the agent is considered the ulti-

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[1]Corresponding author: jreyes@upei.ca

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mate goal and this strategy has been in use since the mid-20th century. For example, in 1933 the first successful eradication program was reported and consisted of a combination of laboratory testing, segregation, and elimination of infected animals (Wilkinson, 1965). Since the beginning of control and eradication programs, the use of penicillin-based products has been recommended (Wilkinson, 1965). *Streptococcus agalactiae* remains highly susceptible to antimicrobials (Makovec and Ruegg, 2003), in particular to β-lactam-based products (Erskine et al., 2002; Edmondson, 2011). Continued sensitivity to antimicrobials has been an important factor in the success of control programs that combine lactational treatment with other recommendations, such as postmilking teat disinfection and dry cow therapy (Keefe, 2012).

The prevalence of mastitis caused by *S. agalactiae* can be successfully reduced with an antimicrobial agent-treatment program and adequate herd management to limit the incidence of new infections. A popular approach for control and eradication is blitz therapy, which is the treatment of all lactating cows simultaneously regardless of infection status; however, this method is commonly modified so that only the culture-positive animals are treated (Edmondson, 2011). The main routes of administration for the treatment of mastitis are intramuscular (**IM**) and intramammary (**IMM**; Sérieys et al., 2005). The selection of treatment route should be made using the following criteria: integrity of the biological barriers of the udder; location of the bacteria in consideration of the physiochemical characteristics of the antimicrobial agent; stage at which the treatment is initiated; and severity of the pathology (Du Preez, 2000). The IMM route is commonly chosen (Du Preez, 2000; Sérieys et al., 2005) and, in this case, the selected antimicrobial agent should exhibit high lipid solubility to allow the product to move through lipid-rich membranes; product efficacy is correlated with the duration of effect in the milk (Gruet et al., 2001). *Streptococcus agalactiae* is sensitive to IMM treatment, as infections in both lactating and dry cows respond to IMM therapy with β-lactam-based products with a cure risk of approximately 90% (Tyler et al., 1992). As a result, treatment by this route will result in the elimination of a high number of infections in a cost-effective manner (Keefe, 1997). The IM route should only be considered for compounds that are highly lipophilic and able to cross the epithelia into the mammary gland parenchyma (Gruet et al., 2001). Additionally, IM products should continue to be active in inflammatory secretions and should achieve effective therapeutic concentrations within the mammary gland (Pyörälä, 2006). The IM route has been reported as effective for the treatment of *S. agalactiae* (Tyler et al., 1992). Across pathogen species, IM treatment has been suggested when more than 1 quarter is affected, in cases of chronic subclinical infections (Barkema et al., 2006), or when the infection is clinical in nature (Pyörälä, 2006).

Several studies have examined cure risk for subclinical *S. agalactiae* and other streptococci infections after either IM or IMM therapy versus either negative controls or other products with the same route of administration. One study compared cure risk for clinical mastitis between IM and IMM treatment and found no difference (Sérieys et al., 2005). However, their study had very few (7 of 312) *S. agalactiae*-associated cases. No studies have focused on a direct comparison of IM and IMM therapy for subclinical *S. agalactiae* infections.

In Colombia, the high prevalence of *S. agalactiae*, combined with climatic factors, hand milking, and variable adoption of hygienic practices makes the control and eradication of *S. agalactiae* particularly challenging. Growing interest from the dairy industry and academia has led to efforts to determine a more efficient treatment strategy for the country-specific herd conditions. This treatment strategy also needs to address the best treatment regimen (administration route, product) that addresses the concerns of the producers.

The present study evaluated the efficacy of 2 products—ampicillin and cloxacillin IMM infusion (Masticillin Lactation, Bayer, Leverkusen, Germany), which was reported to have a robust IMM distribution (Gruet et al., 2001), and IM penethamate injection (Mamyzin P, Boehringer Ingelheim GmbH, Ingelheim, Germany), a weak base lipophilic compound with reported high concentrations of benzylpenicillin in the mammary gland (McDougall, 1998)—for the treatment of *S. agalactiae* in dairy cows from the departments of Antioquia and Caldas, Colombia. The methodology was a randomized clinical trial, controlling for the herd effect and individual SCC with the outcome of cow bacteriological cure.

MATERIALS AND METHODS

Sample Size

The minimum number of cows to be included in the controlled clinical trial was estimated based on methodology for the comparison of 2 proportions using the methods reported by Dohoo et al. (2009), although a more robust hierarchical methodology was eventually used. For the sample size calculation, the following assumptions were made: a 2-sided χ^2 test with α of 0.05 and a power $(1 - \beta)$ of 0.80. We assumed an expected

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