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A non-inferiority study evaluating a new extended-release preparation of tilmicosin injected subcutaneously vs. ceftiofur administered intramammary, as dry-cow therapy in Holstein Friesian cows

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ABSTRACT

Background: A new, extended long-acting tilmicosin (TLAe) preparation was tested against intramammary ceftiofur (CEF) using a non-inferiority trial model during dry-cow therapy (DCT) in a farm with high bovine population density and deficient hygiene application.
Objectives: To evaluate the possibility that TLAe administered parenterally can achieve non-inferiority status compared to CEF administered intramammary for DCT.
Methods: Cows were randomly assigned to TLAe (20 mg/kg subcutaneous; n = 53) or CEF (CEF-HCl, 125 mg/quarter; n = 38 cows) treatment groups. California mastitis testing, colony-forming unit assessment (CFU/mL), and number of cases positive for *Staphylococcus aureus* were quantified before DCT and 7 d after calving. A complete cure was defined as no bacteria isolated; partial cure when CFU/mL ranged from 150 to 700, and cure-failure when CFU/mL was above 700.
Results: TLAe and CEF had overall cure rates of 57% and 53% ($p > 0.05$) and *S. aureus* cure rates of 77.7% and 25%, respectively ($p < 0.05$). The pathogens detected at DCT and 7 days after calving were *S. aureus* (62.71% and 35.55%), *Staphylococcus* spp. (22.03% and 35.55%), *Streptococcus uberis* (10.16% and 13.33%), and *Escherichia coli* (5.08% and 15.55%). Non-inferiority and binary logistic regression analyses revealed a lack of difference in overall efficacies of TLAe and CEF. Apart from *S. aureus*, *S. uberis* was the predominant pathogen found in both groups.
Conclusions: This study is the first successful report of parenteral DCT showing comparable efficacy as CEF, the gold-standard. The extended long-term pharmacokinetic activity of TLAe explains these results.

Keywords: Mastitis, bovine; dry-cow therapy; long-acting tilmicosin; ceftiofur; non-inferiority

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Conflict of Interest

The authors declare that they have no competing interests. The National Autonomous University of Mexico (UNAM), owner of the patent, is open to licensing the novel preparation of the long-acting tilmicosin in test outside Mexico.

Author Contributions

Conceptualization: Sumano H, Posadas E; Formal analysis: Ortega E, Tapia G; Funding acquisition: Sumano H; Investigation: Ortega E, Alfonseca-Silva E, Sumano H; Methodology: Tapia G, Sumano H, Posadas E, Alfonseca-Silva E; Project administration: Sumano H, Posadas E, Ortega E; Writing - original draft: Sumano H, Ortega E, Posadas E, Tapia G, Alfonseca-Silva E.

INTRODUCTION

Bovine intramammary infections (IMIs) cause considerable economic losses due to loss of milk production, use of drugs, milk disposal, veterinary services, and, ultimately, when cows need to be culled [1]. It has been shown that IMIs can be greatly reduced if antibiotic treatment is employed during the dry-cow period. Approximately 50% to 63% of cows receiving no dry-cow therapy (DCT) exhibit IMIs during the dry-cow period [2,3]. Increases in new IMIs when lactation starts in mammary-quarters that did not receive DCT can be as high as 10% to 12% [4]. Infections by *Staphylococcus aureus* and coagulase-positive staphylococci are the main causes of IMIs at the beginning of the lactation cycle [5]. Cure-rate variations depend upon the selected antibiotic, the particular pathogen involved, the individual cow, and the herd. The cow's age, somatic cell count, duration of previous infections, bacterial colony counts in milk before treatment, and the number of infected quarters can also influence the cure rate [6]. Hence, an adequate DCT is of utmost importance to reduce the incidence of IMI at the beginning of lactation. Gruet et al. [7] and Gehring and Smith [8] listed the main antibacterial drugs for DCT, all of which are prepared as intramammary infusions (i.e., ceftiofur [CEF], cloxacillin, penicillin, neomycin, and cephapirin benzathine). These antibiotics can show adequate control of IMI caused by *Streptococcus agalactiae* and sometimes for IMIs caused by *S. aureus* and *Streptococcus uberis* [9]. Experimentally, an intramammary formulation of 1,500 mg of tilmicosin was reported to be equally effective as cephapirin benzathine for eliminating *S. aureus* mastitis [10]. When administered intramammary, the tilmicosin cure rate for *S. aureus* infection in the dry period was similar to that obtained by the administration of cloxacillin [11]. However, Mohammadsadegh [12] reported that intramammary infusion of 1,500 mg tilmicosin for DCT therapy had less effect on reducing IMI due to *Corynebacterium bovis* than that of cloxacillin and had no effect on *S. agalactiae*, and although it had a potent effect against *S. aureus*, the author concluded that tilmicosin alone should not be infused as an alternative to conventional DCT.

In contrast to the almost universal acceptance of intramammary administration of antibiotics during the dry-cow period, parenteral administration is rarely accepted. However, at least theoretically, systemic therapy would achieve a more uniform distribution of the drug in mammary tissue, and if the drug tissue distribution is adequate, effectiveness should be appropriate. Tilmicosin has an excellent distribution in mammary tissue, is rapidly accumulated in bovine macrophages and mammary epithelial cells [13], and possesses high efficacy vs. *Staphylococcus* spp. [14]. Hence its parenteral administration should theoretically be useful for DCT. The MIC 90 value of tilmicosin against 112 *S. aureus* isolates from cows with IMIs was 0.78 μg/mL, and a single subcutaneous (SC) injection of 10 mg/kg of tilmicosin can achieve concentrations suitable for the control of *S. aureus* for 8–9 days [15]. Moreover, the greater susceptibility of these staphylococci to tilmicosin compared with other antibiotics tested *in vitro*, suggests it is an excellent choice for the treatment of IMI [16]. However, contrary to expectations, Owens et al. [17] found low efficacy for DCT (9%) when tilmicosin was injected twice, 4 days apart, at a dose of 5 mg/kg subcutaneously.

There is a new pharmaceutical preparation of tilmicosin available in some countries (patent 212148 in favor of the National Autonomous University of Mexico [UNAM]; Instituto Mexicano de la Protección Industrial, Mexico City, Mexico). The pharmacokinetics of this 39% poloxamer-407-based tilmicosin preparation, injected subcutaneously at a dose of 20 mg/kg, has shown extended long-action that is equivalent to 9–10 days of useful plasma concentrations [18], as well as 20 days of useful concentrations in mammary secretions in

the dry-cow period, against *Staphylococcus* spp. [19]. This pharmacokinetic profile was the impetus to evaluate the clinical efficacy of this pharmaceutical preparation of extended long-acting tilmicosin (TLAe) by injecting it as a single SC dose of 20 mg/kg in a non-inferiority trial setting *vs.* the intramammary administration of CEF (125 mg/quarter). That is, cows injected with TLAe at the time of dry-off should have a non-inferior proportion of animals cured of preexisting IMI after calving compared to cows treated by intramammary administration of CEF.

MATERIALS AND METHODS

Study design and animal handling complied with Mexican prescripts (NOM-062-ZOO-2001). This trial was implemented at a dairy farm intensively producing Holstein/Friesian cows in the Agricultural and Industrial Complex of Tizayuca SA, State of Hidalgo, Mexico, from November 2018 to August 2019. It is important to point out that the chosen farm is contained within an industrial complex of several dairies with high bovine population densities and rather deficient hygiene and bovine management approaches. This scenario was chosen to provide the trial with a more extreme challenge in evaluating the efficacy of TLAe as a treatment for parenteral drying. The trial included 106 clinically healthy cows, each with a range of 2–5 calvings, without a record of being treated with antibiotics 20 days before trial initiation. Before treatment, the California mastitis test (CMT) was used to determine the degree of IMI. The CMT estimates the somatic cell count (SCC) of milk samples and determines IMI degree as follows: 0 degree (normal, SCC < 2×10^5 cells/mL); slight (trace) degree of subclinical mastitis (SCC; 1.5–5 × 10^5 cells/mL); 1+ degree (4–15 × 10^5 cells/mL); 2+ degree ($8 \times 10^5 - 5 \times 10^6$ cells/mL); and 3+ degree (SCC > 5×10^6 cells/mL) [20]. This assessment was repeated every other day after calving for 30 days. The prevalence of mastitis in the chosen dairy, quantified by individual mammary gland quarter, and as revealed by CMT results, is shown in **Fig. 1**. Negative gland-quarter results ranged from 60% to 68%, with 16% to 22% as CMT degree 1; 2.2% to 9.1% as degree 2, and 5% to 10% as degree 3.

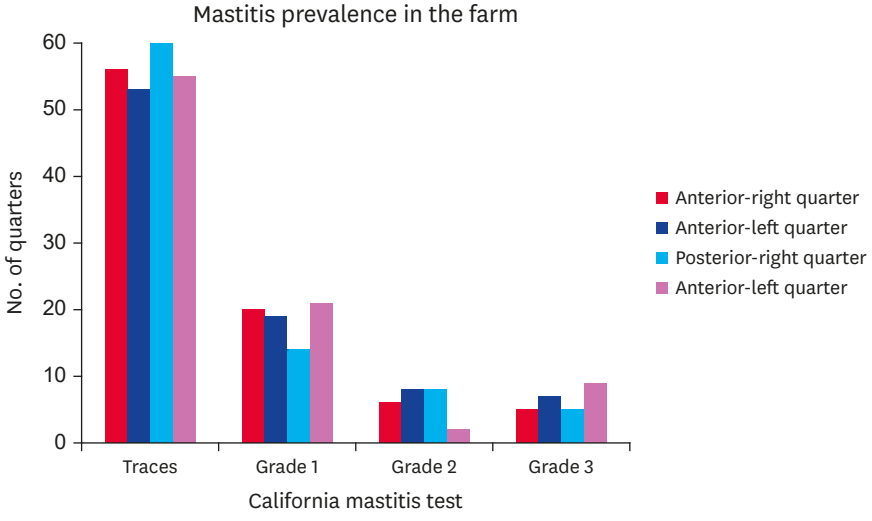

Fig. 1. Quantified by individual gland-quarter, and as revealed by the California mastitis test.

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The cows were randomly distributed to 2 groups. The experimental group (n = 53) was treated with a single 20 mg/kg dose of TLAe (Karitil-Premium 39%®, Karizoo, Mexico; based on patent 212148 in favor of UNAM) injected subcutaneously at 3 different injection sites on the day the cow entered the DCT program. The dose was administered on the lateral sides of the neck adjacent to the cranial part of the shoulder using 20 mL syringes and 18-gauge hypodermic needles, delivering no more than 10 mL of the dose per injection site. The other group (n = 38 cows) was treated with 125 mg of CEF (Spectramast; Zoetis, Mexico City, Mexico) administered through the intramammary route. The administration of this preparation was carried out after cleaning and disinfecting the nipples with iodine-polyvinylpyrrolidone for 25–30 sec, followed by careful disinfection of the nipple-tip with 70% ethyl alcohol.

For both groups, and before the last milking of the lactation period, 20 mL of milk was collected from each mammary gland following the guidelines of the National Mastitis Council [21] and utilizing sterile 50 mL tubes with caps. Upon collection, the tubes were immediately sent for microbiological analysis (transported at approximately 4°C) at the Department of Microbiology and Immunology of the School of Veterinary Medicine, UNAM. The same procedure was repeated within 72 h after calving. For analysis, milk samples were incubated at 37°C for 30 min to detach microorganisms from fat. Each sample was vortexed for 5 min to achieve a homogeneous mixture. In duplicates, 20 μL samples were seeded using the striatum technique on blood agar, MacConkey agar and mannitol salt media plates and incubated under aerobic conditions at 37°C for 24–48 h. Bacteria colonies were initially identified based on the results of gram staining and their morphological and physiological characteristics. The microorganisms were then fully identified using the method described by Carter and Cole [22]. A milk sample was defined as coming from a cow with IMI when the bacterial isolation was ≥ 3 colony-forming units (CFU) for every 20 μL of inoculum. An IMI was considered cured when the previously identified bacterium(a) was not cultured from a sample obtained 7 days after calving. A partial IMI cure was considered if the milk sample after calving showed at least a 50% reduction in CFU/mL from that at the end of the lactation period.

Statistical analysis

The effectiveness of the treatment was determined for each mammary gland that had a bacteriological cure (positive response) following treatment with TLAe or CEF. A multivariate binary logistic regression model was applied to predict clinical efficacy and identify relationships between treatment and other factors that could affect the response to the treatment. The factors examined were cow age, CMT result (a scale to determine udder health), cow body condition, and time of year. The dependent variable had a binary response (1 = positive; 2 = negative), based on the bacteriological and clinical cure. The statistical tests were performed using the IBM SPSS ver-19® statistical package [23].

For certain trials, the objective is to demonstrate that a given treatment is clinically not inferior to or no worse than another treatment. Once an existing therapy has been established, it may no longer be ethical to start placebo-controlled trials; instead, active-controlled trials can be conducted in which a novel treatment is compared with an established treatment [24]. The minimum difference in cure rate to declare non-inferiority of TLAe, as compared with CEF, was pre-stated at 20%. To demonstrate non-inferiority, a total of 92 cows, with an initial enrollment of 106 animals (53 for TLAe and 38 for CEF), were estimated to be required assuming r = 1.5 in favor of group TLAe $\sigma^2 = 0.052$ [24], where $\alpha = 0.05$ and $\beta = 0.2$ plus 15% to adjust for within-herd clustering of cows, and assuming

that 10% of the cows would be infected at dry-off and therefore available for a cure (total enrollment 106 cows). The equation used was:

$$n_1 = \frac{\left[Z_\alpha\sqrt{(r+1)\bar{P}\bar{Q}} + Z_\beta\sqrt{(rP_1Q_1 + P_2Q_2}\right]^2}{r\delta^2}; \; n_2 = \frac{n_1}{4}\left[1 + \sqrt{1 + \frac{2(r+1)}{rn_1\delta}}\right]^2$$

Where $\bar{P} = \frac{P_1 + P_2}{r+1}$ and $\bar{Q} = 1 - \bar{P}$, Z_α, Z_β are the upper 100 (1-p) percentiles of the standard normal distribution, α is the (significance level), β is (1-power of the test), P1and P2 are the proportions expected for each group, r is the allocation ratio, d is the non-inferiority limit $\delta = |P_1 - P_2|$, the non inferiority level [25], being α = 0.05, β = 0.20, P1 = 0.99 for CEF and P2 = 0.79 for TLAe. Tests comparing 2 proportions were analyzed by using MedCalc statistical software (ver. 2020), and *p* values < 0.05 were considered statistically significant.

A total of 106 cows were used to evaluate the risk for IMI after calving. From the 106 cows originally enrolled (TLAe = 65; CEF = 41), 7 cows were excluded because they were culled from the herd during the dry period, 6 cows that calved prematurely (before meeting the 60 day dry period set in this trial) were excluded, and 2 cows were excluded because the post-calving sample was improperly collected and maintained. After those eliminations, 91 cows were included in the trial.

RESULTS

Isolates and percentages of bacteria identified on the first milk sampling just before DCT in the TLAe and CEF groups are presented in **Table 1**. Total and partial bacteriological percentage cure efficacies are also summarized in **Table 1**. The total bacteriological cure rate achieved in the TLAe group was 57% while the reference group (CEF) achieved a bacteriological cure rate of 53%. In both instances, the predominant bacterium detected was *S. uberis* (present in 75% and 100% of the non-cured cases). Differences in the bacteriological cure rate in cows that were positive for *S. aureus* before and after treatment are presented in a contingency table (determined by Pearson χ^2 analysis; **Table 2**). In group TLAe 18 of 46 cases were recorded as *S. aureus*-positive before DCT, and bacteriological cures were achieved in 14 cows (77.7%; $p = 0.371$). In the CEF group, 8 of 30 cases were *S. aureus*-positive at DCT, and a bacteriological cure was obtained in only 2 cows (25%; $p = 0.013$).

Table 1. Isolation and percentage of bacteria identified on the 1st sampling just before DCT either with the parenteral injection of TLAe or with intramammary ceftiofur (125 mg/quarter) (CEF)

Group and X ± SD calvings*	Bacteria	% isolations at DCT	% 7 days after calving	% Efficacy bacteriological			
				Total†	Partial‡	Total + partial§	Clinical‖
TLAe (n = 53, 2.48 ± 0.8)	*S. aureus*	61.1 (n = 22)	28 (n = 7)	45.45 (n = 10)	9.09 (n = 2)	54.5 (n = 12)	57
	Staphylococcus spp.	22.2 (n = 8)	48 (n = 12)	62.5 (n = 5)	0 (n = 0)	62.5 (n = 5)	
	S. uberis	11.11 (n = 4)	12 (n = 3)	50 (n = 2)	0	50	
	E. coli	2.7 (n = 1)	12 (n = 3)	0	0	0	
	Bacillus spp.	2.7 (n = 1)	0 (n = 0)	0	0	0	
CEF (n = 38, 2.47 ± 0.8)	*S. aureus*	58.3 (n = 14)	42.10 (n = 8)	28.5 (n = 4)	14.2 (n = 2)	42.85 (n= 6)	53
	Staphylococcus spp.	22.7 (n = 5)	45 (n = 9)	80 (n = 4)	0	80 (n = 4)	
	S. uberis	4.54 (n = 1)	10 (n = 2)	0	0	0	
	E. coli	9.09 (n = 2)	5 (n = 1)	0	0	0	

In all, 91 samples were studied, and 59 bacteria isolated before DCT and 91 samples were obtained and 45 pathogens isolated 7 days after calving.
DCT, dry-cow therapy; SD, standard deviation; TLAe, extended long-acting tilmicosin; CEF, ceftiofur.
*Range from 1–4; †No isolation could be detected; ‡At least 50% reduction in the number of CFU/mL isolated; §Sum of bacteriological efficacies; ‖Clinical efficacy based on the fact that cows were incorporated into productive milking.

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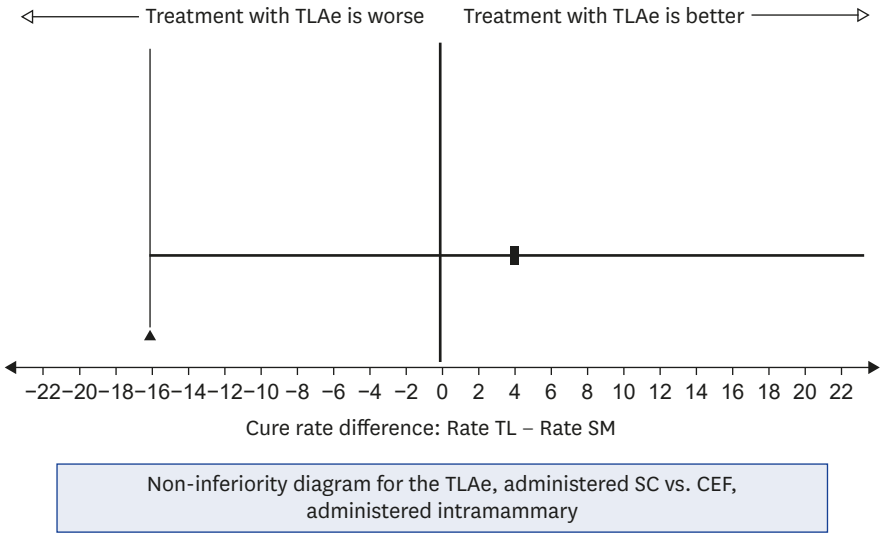

Fig. 2. A non-inferiority study comparing 2 dry-cow therapies. Differences in cure rate (4% [−16.0246%, 23.7779%]) between tilmicosin (TLAe; 56% cured) and CEF (52% cured) in the non-inferiority trial, where the critical difference (Δ) is shown relative to the observed difference and associated 95% confidence interval. TLAe, extended long-acting tilmicosin; SC, subcutaneous; CEF, ceftiofur.

The non-inferiority quality of TLAe was evaluated in 53 cows and a positive treatment response in 56% of the cows was slightly higher than the response to CEF treatment (52%); regardless, the 4% difference between treatments was not statistically significant ($p = 0.7071$; 95% confidence interval, −16.0246%, 23.7779%) (**Fig. 2**).

The logistic regression model used allowed the evaluation of factors affecting treatment efficacy (cured = 1; not cured = 2), such as cow body condition, number of calvings, and CMT result. The analyses showed no significant association between those factors and the treatments applied ($p = 0.676$). However, 2 factors shown to affect the efficacy of the treatments were trace CMT results ($p = 0.018$) and grade 1 CMT results (subclinical mastitis); $p = 0.003$, as shown in **Table 3**. Considering these results, further testing was performed to examine the difference in the efficacies of the treatments within the trace and grade 1 CMT categories. Thus, of the 24 cows with trace CMT results after TLAe treatment, 20 responded positively (83% efficacy). In contrast, of the 21 CEF-treated cows with trace CMT results, 14 exhibited a positive response (66% efficacy). A similar analysis revealed that from 18 TLAe-

Table 2. Contingency table showing *p* values for the efficacy comparison, through Pearson χ^2 analysis, of the dry-cow therapy with TLAe injected subcutaneously at a dose of 20 mg/kg and ceftiofur HCl, administered through the intramammary route (CEF)

Treatment	TLAe	CEF
Before	Negative (28)	Negative (22)
	Positive (18)	Positive (8)
After	Negative (3†)	Negative (2†)
	Positive (4‡)	Positive (6‡)
p value*	0.371	0.013

Negative values represent samples without bacterial growth or when another different microorganism from *Staphylococcus aureus* was detected. Positive values mean *S. aureus* positive samples.
TLAe, extended long-acting tilmicosin; CEF, ceftiofur.
*χ^2 likelihood ratio test; †New intramammary infection, considering that at dry-cow therapy they were *S. aureus*-free; ‡Not cured.

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Table 3. Results of the logistic regression model to evaluate the effect of the factors to the efficacy of the treatments

Variables	B	SE	Wald	df	Sig.	Exp(B)	95% CI for EXP(B)	
							Lower	Higher
Treatment (1)	−0.214	0.511	0.175	1	0.676	0.808	0.297	2.200
Parity			0.305	2	0.858			
Parity (1)	0.128	0.705	0.033	1	0.856	1.136	0.285	4.528
Parity (2)	−0.227	0.550	0.170	1	0.680	0.797	0.271	2.342
Body Cond	1.209	1.274	0.900	1	0.343	3.349	0.276	40.702
California			10.459	4	0.033			
California (1)	−1.353	0.572	5.598	1	0.018	0.258	0.084	0.793
California (2)	−2.083	0.711	8.581	1	0.003	0.125	0.031	0.502
California (3)	−22.004	17922.391	0.000	1	0.999	0.000	0.000	
California (4)	−22.419	28420.600	0.000	1	0.999	0.000	0.000	
Constant	−2.925	4.529	0.417	1	0.518	0.054		

Variables included: parity (number of calvings per cow), body condition (1–5 covariate), and California mastitis test (4 categories: 0, 1, 2, 3).
SE, standard error; df, degree of freedom; Sig., significance; CI, confidence interval.

Table 4. Proportion difference test for cows in three different subgroups as follows: CMT scored traces, CMT scored 1, and sum of both subgroups

Statistical parameters	Subgroups		
	CMT-traces	CMT – 1	Sum of CMT traces + 1
Differences (substraction value)	83–66% (17%)	55–28% (27%)	71–57% (14%)
95% CI	−8.22, 40.55	−14.7551, 55.3184	−8.2131, 78
Ji-square	1.691	1.415	1.434
df	1	1	1
Level of significance	$p = 0.1935$	$p = 0.2343$	$p = 0.2311$

MedCalc uses the "N–1" χ^2 test.
CMT, California mastitis test; CI, confidence interval; df, degree of freedom.

treated cows with grade1 CMT results had an efficacy of 55%, while CEF-treated cows had only a 28% efficacy. By combining the results for the trace and grade 1 subgroups, the percent efficacy for TLAe treatment was 71% and that for CEF was 57% (**Table 4**).

DISCUSSION

It should be noted that the clinical scenario used for this study was an important factor in obtaining the cure rates observed. The location chosen, in the Tizayuca-Complex in the State of Hidalgo, Mexico, is a challenging one because in this dairy-complex, hygiene measures are limited and antimicrobial resistance predictably high [26]. For example: in addition to an overpopulation of milk-producing cattle concentrated in an insufficiently extended region, there are not enough sanitary fences and fords, and the disinfectant used is not replaced regularly. Moreover, milking equipment and facility cleaning routines are deficient, excreta management is faulty, and fly control is scarce if not lacking. Thus, it has been recognized that there are important sanitary management issues to address in this region [27]. For example, a study assessing the quality of milk from this region against national and international standards concluded that the milk produced in this region was of excellent physicochemical quality (fat and non-fat solids); however, it was described as poor quality due to its SCCs and poor sanitation standards [28]. It has also been suggested that it is necessary to promote the incorporation and enforcement of quality and safety concepts in this region [29]. Despite these factors, the success rate in the bacteriological cure assessment of the new pharmaceutical preparation of tilmicosin was 77.7% compared to only 25% success for intramammary CEF-HCl.

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Most studies agree that the dry-cow period is a risk factor that can increase susceptibility for the acquisition of new IMIs. To keep cow mammary glands healthy, it is widely recognized that the administration of an antimicrobial drug, such as DCT, is necessary [30,31]. Such a practice is intended to prevent new infections and eliminate subclinical ones. Although many bacteria can cause IMIs in the dry-cow period, the main etiologic agent is *S. aureus*, while toward the end of the dry-cow period it is *S. uberis* [32]. In particular, IMI caused by *S. aureus* is considered one of the major mastitis pathogens affecting dairies worldwide. The primary method of spread for these pathogens is from cow to cow. However, there is much to elucidate about the epidemiology of *S. aureus* IMIs, and this lack may be related to the lack of efficient control of *S. aureus* IMI [33,34]. Additionally, *S. aureus* has a high level of resistance to antibacterial drugs, and its resilience is also associated with its ability to form biofilms within mammary tissue. These features explain the poor bacteriological cure rates associated with most antibacterial therapies and the occurrence of relapse. It has been suggested that long-acting antibiotic preparations administered at drying off can be effective [35,36]; however, their efficacy is generally described as poor and variable [11,26]. Thus, the administration of antibacterial drugs for DCT is carried out almost exclusively through the intramammary route. However, parenteral administration of some agents has been attempted. For example, Nickerson and Owens [10] administered tilmicosin (10 mg/kg SC) and obtained a bacteriological cure rate against *S. aureus* of only 9.1%. In contrast, in this study, a much higher bacteriological cure rate (77.77%) was observed with a 20 mg/kg dose of an long-acting preparation of tilmicosin, which maintains therapeutic concentrations in plasma for up to 10 days and in mammary gland fluids for up to 20 days during the dry period [18]. In other studies in which parenteral DCT was attempted, even better efficacy percentages, approaching complete efficacy, have been reported for the control of *S. aureus* [19]. However, for tilmicosin, intramammary administration seems to provides better results than those from parenteral administration. Dingwell et al. [11] used an infusion of tilmicosin of 1,500 mg per mammary quarter and achieved a general bacteriological cure rate of 67.3%. Mohammadsadegh [12], also infusing 1,500 mg of tilmicosin per quarter, reported a bacteriological cure rate greater than 80% for *S. aureus* and 87% for coagulase-negative staphylococci.

Results in this study show sufficient efficacy against IMI by *S. aureus* when using a parenteral administration of a specific long-action formulation of tilmicosin at 20 mg/kg, producing a cure rate of 77.7%. This result may reflect an intrinsic efficacy of this macrolide drug against *S. aureus* and the enhanced pharmacokinetics of the tested preparation of tilmicosin, as well it reflects an increase in dosage rose from 10 to 20 mg/kg [18,19]. It is also important to note that approximately 50% of the cows included in this study ranged from their 1st to 3rd calving and the rest ranged from their 4th to 5th calving. The former cows responded better than the latter, which has been observed previously [37,38].

Although it has been proposed that bacteriological cure status should be assessed at 28 days after calving, conditions in the farm in this study were too challenging. To reduce the effects of environmental contamination at the farm, it was deemed appropriate to assess antibacterial efficacy vs. *S. aureus* at 7 days after calving [37]. That decision was also based on the results in a study that evaluated the sensitivity and specificity of the diagnosis of IMI using only one sample *vs.* triplicate samples obtained on different days in which an almost negligible gain was achieved by triplicate sampling either in specificity or sensitivity to diagnose IMI [38]. Some factors that may have contributed to the number of non-cured, *S. aureus*-infected cows in this trial include the chronicity of the active infection, the number of

CFUs in milk before undertaking DCT, and the number of infected mammary quarters [32]. It has been reported that when a quarter is not cured in a cow, self-infection of the adjacent quarters is relatively easy [39,40].

The effect of both treatments on the CMT results reflected the microbiological assessment results (p = 0.039 and 0.003 for overall and *S. aureus* efficacy, respectively). Apart from the treatment-failures due to *S. aureus* infection, the predominant pathogen detected in both groups was *S. uberis*. It is important to emphasize that *S. uberis* is a pathogen that shows increased pathogenicity toward the end of the dry period [38,40,41]; thus, strategic administration of other antibacterial drugs during that period should be studied. *In vitro* culture of *S. uberis* is relatively dependable when using standard laboratory techniques. Therefore, it is hardly feasible to assume that reinfections by this pathogen were caused by diagnostic flaws at the beginning of this trial [40,42]. Hence, IMI after calving is regarded as an infection that is acquired during the dry-cow period.

In conclusion, this is perhaps the first report of a parenteral treatment intended for DCT that can be regarded as successful. The results indicate that DCT using parenteral administration of TLAe is a viable treatment option and an acceptable alternative to CEF treatment, given that the non-inferiority assessment revealed a lack of statistically significant differences between these 2 antibacterial treatment options. Also, it is worth emphasizing that TLAe was particularly efficient in the treatment of intramammary *Staphylococcus* spp. infections during the dry-cow period.

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REFERENCES

1. Hogeveen H. Mastitis is an economic problem. In: Proceedings of the British Mastitis Conference; October 12, 2005, Warwickshire, UK.
2. Neave FK, Dodd FH, Kingwill RG, Westgarth DR. Control of mastitis in the dairy herd by hygiene and management. J Dairy Sci. 1969;52(5):696-707.
PUBMED | CROSSREF
3. Oliver SP. Frequency of isolation of environmental mastitis-causing pathogens and incidence of new intramammary infection during the nonlactating period. Am J Vet Res. 1988;49(11):1789-1793.
PUBMED
4. Eberhart RJ. Management of dry cows to reduce mastitis. J Dairy Sci. 1986;69(6):1721-1732.
PUBMED | CROSSREF
5. Green MJ, Bradley AJ, Medley GF, Browne WJ. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. J Dairy Sci. 2007;90(8):3764-3776.
PUBMED | CROSSREF
6. Sol J, Sampimon OC, Snoep JJ, Schukken YH. Factors associated with bacteriological cure after dry cow treatment of subclinical staphylococcal mastitis with antibiotics. J Dairy Sci. 1994;77(1):75-79.
PUBMED | CROSSREF
7. Gruet P, Maincent P, Berthelot X, Kaltsatos V. Bovine mastitis and intramammary drug delivery: review and perspectives. Adv Drug Deliv Rev. 2001;50(3):245-259.
PUBMED | CROSSREF

8. Gehring R, Smith GW. An overview of factors affecting the disposition of intramammary preparations used to treat bovine mastitis. J Vet Pharmacol Ther. 2006;29(4):237-241.
PUBMED | CROSSREF
9. Erskine RJ, Wagner S, DeGraves FJ. Mastitis therapy and pharmacology. Vet Clin North Am Food Anim Pract. 2003;19(1):109-138.
PUBMED | CROSSREF
10. Nickerson SC, Owens WE, Fox LK, Scheifinger CC, Shryock TR, Spike TE. Comparison of tilmicosin and cephapirin as therapeutics for *Staphylococcus aureus* mastitis at dry-off. J Dairy Sci. 1999;82(4):696-703.
PUBMED | CROSSREF
11. Dingwell RT, Leslie KE, Duffield TF, Schukken YH, DesCoteaux L, Keefe GP, et al. Efficacy of intramammary tilmicosin and risk factors for cure of *Staphylococcus aureus* infection in the dry period. J Dairy Sci. 2003;86(1):159-168.
PUBMED | CROSSREF
12. Mohammadsadegh M. Impact of intramammary tilmicosin infusion as a dry cow therapy. J Vet Pharmacol Ther. 2018;41(1):22-27.
PUBMED | CROSSREF
13. Scorneaux B, Shryock TR. Intracellular accumulation, subcellular distribution, and efflux of tilmicosin in bovine mammary, blood, and lung cells. J Dairy Sci. 1999;82(6):1202-1212.
PUBMED | CROSSREF
14. Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, et al. A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. mBio. 2013;4(1):e00537-e12.
PUBMED | CROSSREF
15. Ziv G, Shem-Tov M, Glickman A, Winkler M, Saran A. Tilmicosin antibacterial activity and pharmacokinetics in cows. J Vet Pharmacol Ther. 1995;18(5):340-345.
PUBMED | CROSSREF
16. Naccari F, Martino D, Giofrè F, Passantino A, De Montis P. Therapeutic efficacy of tilmicosin in ovine mammary infections. Small Rumin Res. 2003;47(1):1-9.
CROSSREF
17. Owens WE, Nickerson SC, Ray CH. Efficacy of parenterally or intramammarily administered tilmicosin or ceftiofur against *Staphylococcus aureus* mastitis during lactation. J Dairy Sci. 1999;82(3):645-647.
PUBMED | CROSSREF
18. Gutiérrez L, Soriano R, Martínez-Cortés I, Miranda-Calderon J, Sumano H. Pharmacokinetics of a new parenteral formulation of tilmicosin-la in cows. Pak Vet J. 2016;36:165-168.
19. Mendoza J, Martínez-Cortés I, López-Ordaz R, Gutiérrez L, Sumano H. Concentrations of tilmicosin in mammary gland secretions of dairy cows following subcutaneous administration of one or two doses of an experimental preparation of tilmicosin and its efficacy against intramammary infections caused by *Staphylococcus aureus*. Am J Vet Res. 2016;77(9):922-930.
PUBMED | CROSSREF
20. Kivaria FM, Noordhuizen JP, Nielen M. Interpretation of California mastitis test scores using *Staphylococcus aureus* culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dar es Salaam region of Tanzania. Prev Vet Med. 2007;78(3-4):274-285.
PUBMED | CROSSREF
21. Procedures for Collecting Milk Samples [Internet]. New Prague: National Mastitis Council; http://www.nmconline.org. Updated 2004. Accessed 2019 Aug.
22. Carter G, Cole J Jr. Diagnostic Procedures in Veterinary Bacteriology and Mycology. 5th ed. San Diego: Academic Press; 1991, p 620.
23. IBM Corporation. IBM SPSS Statistics Base 20. Armonk: IBM Corporation; 2011.
24. Johnson AP, Godden SM, Royster E, Zuidhof S, Miller B, Sorg J. Randomized noninferiority study evaluating the efficacy of 2 commercial dry cow mastitis formulations. J Dairy Sci. 2016;99(1):593-607.
PUBMED | CROSSREF
25. Flight L, Julious SA. Practical guide to sample size calculations: non-inferiority and equivalence trials. Pharm Stat. 2016;15(1):80-89.
PUBMED | CROSSREF
26. Samaniego-Barrón ML, Contreras JJL, Jaramillo-Arango CJ, Aguilar-Romero F, Navarrete JV, Hernández-Castro R, et al. Antimicrobial resistance in Mannheimia haemolytica strains isolated from dairy cattle nasal exudate. Vet Mex. 2012;43(2):123-132.
PUBMED | CROSSREF

27. Xolalpa VM, Pérez-Ruano M, Garcia C. Factores asociados a eventos de falla reproductiva de los bovinos hembras del Complejo Agropecuario e Industrial de Tizayuca (CAITSA), Hidalgo México, durante el período de 2000 a 2001. Rev Salud Anim. 2003;25(2):129-137.
28. Cervantes-Escoto F, Cesín-Vargas A, Mamani-Oño I. Standard quality of milk in the State of Hidalgo, México. Rev Mex De Cienc Pecu. 2013;4(1):75-86.
29. Cuevas-Reyesa V, Espinosa-García JA, Flores-Mendiola AB, Romero-Santilland F, Vélez-Izquierdo A, Jolalpa-Barrera JL, et al. A diagnosis of the milk agrifood chain in the State of Hidalgo. Téc Pecu Méx. 2007;45(1):25-40.
30. Dodd FH, Westgarth DR, Neave FK, Kingwill RG. Mastitis--the strategy of control. J Dairy Sci. 1969;52(5):689-695.
PUBMED | CROSSREF
31. Natzke RP, Everett RW, Bray DR. Effect of drying off practices on mastitis infection. J Dairy Sci. 1975;58(12):1828-1835.
PUBMED | CROSSREF
32. Jayarao BM, Gillespie BE, Lewis MJ, Dowlen HH, Oliver SP. Epidemiology of *Streptococcus uberis* intramammary infections in a dairy herd. Zentralbl Veterinarmed B. 1999;46(7):433-442.
PUBMED | CROSSREF
33. Keefe G. Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. Vet Clin North Am Food Anim Pract. 2012;28(2):203-216.
PUBMED | CROSSREF
34. Zecconi A. *Staphylococcus aureus* mastitis: what we need to know to control them. Isr J Vet Med. 2010;65:93-99.
35. Nickerson SC, Ryman VE. Antibiotic therapy in mastitis control for lactating and dry cows. University of Georgia. Extension. Bulletin 1516, May 2019. https://secure.caes.uga.edu/extension/publications/files/pdf/B%201516_1.PDF. Updated 2020. Accessed 2020 Aug 27.
36. Kerro Dego O, van Dijk JE, Nederbragt H. Factors involved in the early pathogenesis of bovine *Staphylococcus aureus* mastitis with emphasis on bacterial adhesion and invasion. A review. Vet Q. 2002;24(4):181-198.
PUBMED | CROSSREF
37. McDougall S, Agnew KE, Cursons R, Hou XX, Compton CR. Parenteral treatment of clinical mastitis with tylosin base or penethamate hydriodide in dairy cattle. J Dairy Sci. 2007;90(2):779-789.
PUBMED | CROSSREF
38. Barkema HW, Schukken YH, Zadoks RN. Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. J Dairy Sci. 2006;89(6):1877-1895.
PUBMED | CROSSREF
39. Newbould FH. Antibiotic treatment of experimental *Staphylococcus aureus* infections of the bovine mammary gland. Can J Comp Med. 1974;38(4):411-416.
PUBMED
40. Zadoks RN, Allore HG, Barkema HW, Sampimon OC, Wellenberg GJ, Gröhn YT, et al. Cow- and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. J Dairy Sci. 2001;84(12):2649-2663.
PUBMED | CROSSREF
41. Berry EA, Hillerton JE. The effect of selective dry cow treatment on new intramammary infections. J Dairy Sci. 2002;85(1):112-121.
PUBMED | CROSSREF
42. Dohoo IR, Smith J, Andersen S, Kelton DF, Godden S; Mastitis Research Workers' Conference. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J Dairy Sci. 2011;94(1):250-261.
PUBMED | CROSSREF