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Economic impact of subclinical mastitis treatment in early lactation using intramammary nisin

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ABSTRACT

Treatment of subclinical mastitis (SCM) during lactation is rarely recommended due to concerns related to both antimicrobial usage and the costs associated with milk discard. Nisin is a naturally produced antimicrobial peptide with a gram-positive spectrum that, when given to dairy cows, does not require milk discard. We evaluated the economic impact of the treatment of SCM during early lactation using a nisin-based intramammary treatment under different scenarios that included various treatment costs, milk prices, and cure rates. We stochastically simulated the dynamics of SCM detected during the first week of lactation. The net economic impact was expressed in US dollars per case. The probabilities of an event and their related costs were estimated using a model that was based on pathogen-specific assumptions selected from peer-reviewed articles. Nisin cure rates were based on results of pivotal studies included in the US Food and Drug Administration (FDA) approval submission. Based on our model, the average cost of a case of intramammary infection (i.e., only true-positive cases) in early lactation was $170 (90% = $148–$187), whereas the cost of a clinical mastitis case was $521 (90% range = $435–$581). Both estimates varied with etiology, parity, and stage of lactation. When comparing the net cost of SCM cases (i.e., CMT-positive tests) detected during the first week of lactation, nisin treatment generated an average positive economic impact of $19 per CMT-positive case. The use of nisin to treat SCM was beneficial 93% of the time. Based on the sensitivity analysis, treatment would result in an economically beneficial outcome for 95% and 73% of multiparous and primiparous cows, respectively. At the herd level, use of intramammary nisin to treat SCM in cows in early lactation was economically beneficial in most tested scenarios. However, the economic impact was highly influenced by factors such as rate of bacteriological cure, cost of treatment, and parity of the affected animal. These factors should be considered when deciding to use nisin as a treatment for SCM.
Key words: subclinical mastitis, treatment cost, nisin

INTRODUCTION

Although SCM is often unnoticed, it is a costly disease for the dairy industry (Ruegg, 2018; Hogeveen et al., 2019). Costs of SCM include reductions in milk quality and quantity (Halasa et al., 2007; van den Borne et al., 2011), an increased likelihood of clinical mastitis, and the potential for transmission to other cows (van den Borne et al., 2010; Barlow et al., 2013). To reduce the impact of SCM, prevention strategies include the application of teat disinfectant during milking, antibiotic treatments, and segregation or removal of infected cows (Ruegg, 2018).

The purpose of antibiotic treatments is to enhance bacteriological clearance which leads to a reduction in the duration of infection and prevention of new infections (Swinkels et al., 2005; Barlow et al., 2009; Down et al., 2013). The benefit of treating SCM is dependent on both farm-specific (e.g., the economic value of milk, replacement heifers) and cow-specific factors (e.g., day of diagnosis, duration of infection, transmission, and cure rates; Halasa et al., 2007; Barlow et al., 2009; McDougall et al., 2022). On most farms, antibiotic treatments for SCM are given at the end of the lactation as IMM dry-cow therapy. Previous studies have cited the cost of milk discard as the main limiting factor for the treatment of SCM during lactation, which has not been offset by potential benefits associated with bacteriological clearance (Swinkels et al., 2005; Steeneveld et al., 2007; van den Borne et al., 2010).

Nisin is a natural antimicrobial peptide of 34 amino acids produced by *Lactococcus lactis* (Carr et al., 2002). Nisin is generally recognized as safe for human consumption (Federal Register, 2011) and is used as a food preservative (Khelissa et al., 2021; Verma et al., 2022). Nisin acts by inhibition of peptidoglycan biosynthesis

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and formation of membrane pores (Field et al., 2008; Chai et al., 2015; Chernyshova et al., 2022). It is effective against a wide range of gram-positive bacteria (Sears et al., 1992; Field et al., 2012, 2021), which account for the majority of pathogens that cause SCM (Middleton et al., 2004; Rowe et al., 2020). The efficacy of nisin against cases of clinical and subclinical mastitis has been evaluated with promising results (Cao et al., 2007; Wu et al., 2007). A nisin-based IMM formulation (Re-Tain, ImmuCell, Portland, ME) labeled for the treatment of SCM is under review by the US Food and Drug Administration (**FDA**) for use to treat SCM, and no milk discard will be required. The economic impact of treatment of SCM during lactation using a product that does not require discard of milk has not been previously evaluated. This study aimed to evaluate the economic impact of IMM treatment of subclinical mastitis in early lactation cows using nisin, and to determine the economic impact of the intervention under different scenarios.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Model Overview

The study was conducted using a stochastic Monte Carlo simulation model and decision tree that we developed in Microsoft Excel using @Risk 7.6 and PrecisionTree software (Palisade Corporation, Ithaca, NY). Model outcomes were generated in 3 steps. First, we modeled the probabilities of events associated with the diagnosis of SCM in a single mammary gland using the CMT)to detect SCM at 7 DIM in primiparous and multiparous cows. In the second step, we modeled the probabilities associated with nisin treatment of SCM cases. The third step involved the allocation of cost to each SCM event and comparison of the total costs based on treatment or no treatment.

The probability of events and their associated costs were stochastically estimated using randomly generated numbers drawn from relevant distributions. These distributions were derived from available databases and peer-reviewed journal articles. Supplemental Table S1 (see Notes) provides probability distribution and references used through the stochastic simulation model. Model simulations were run using 10,000 iterations to generate stable results. The outcomes of each iteration were combined to form an outcome distribution that accounts for the natural variation of the biological system (Hogeveen et al., 2019; Bonestroo et al., 2023). The outcomes reported from the model are the average cost per case of SCM within a lactation (in US dollars) under both treatment strategies and the difference in costs between treatment strategies (i.e., economic net impact) based on expected values from a commercial dairy herd in the United States with 1,000 lactating cows per year (Table 1).

Dynamics of Pathogen-Specific Infection

The dynamics of SCM were modeled based on expected probabilities (Figure 1). A case of SCM was defined as a cow having a CMT score of "trace" or higher (NMC, 2017) in a single quarter after testing between 4 and 10 DIM. In line with the approach used by Bonestroo et al. (2023), only single-quarter SCM cases were considered due to the limited amount of literature to simulate the outcomes resulting from interactions of multiple SCM cases. When treatment followed diagnosis of SCM, it involved infusion of 3 IMM applications of 30 mg of nisin at 12-h intervals (Re-Tain, ImmuCell; Portland, ME) in the CMT-positive quarter. Untreated cases did not receive any intervention.

Etiologies were categorized as (1) NAS, (2) *Streptococcus* spp. and *Streptococcus*-like organisms (**SSLO**), (3) *Staphylococcus aureus*, (4) other gram-positive pathogens (e.g., *Actinomyces* spp., *Bacillus* spp., *Corynebacterium* spp.), (5) coliforms (*Escherichia coli*, *Klebsiella pneumoniae*), and (6) other gram-negative pathogens (*Pseudomonas* spp., *Serratia* spp.). The distribution of etiologies was based on Godden et al. (2017) and Rowe et al. (2020).

Bacteriological cures attributable to nisin for NAS, SSLO, and other gram-positive pathogens were esti-

Table 1. Values of the base herd used in the pathogen-specific stochastic model

Variable	Distribution	Values[1]	Source
Replacement rate (culling rate)	Pert	0.32, 0.37, 0.44	Leite de Campos et al., 2021
Calving interval (d)	Pert	328, 396, 488	USDA, 2014
Dry period length (d)	Pert	38, 53, 68	USDA, 2014
Lactation length (d)	Pert	314, 343, 431	USDA, 2014
Milk yield 305d (kg/cow) healthy primiparous cows	Normal	10, 993, 1,000	Adapted from Wood, 1967
Milk yield 305d (kg/cow) healthy multiparous cows	Normal	12, 165, 1,000	Adapted from Wood, 1967
IMI prevalence first week (%)	Normal	25.2, 3.0	Godden et al., 2017; Rowe et al., 2020

[1]Values are expressed based on the following distributions: pert (minimum, mode, maximum) or normal (mean, SD).

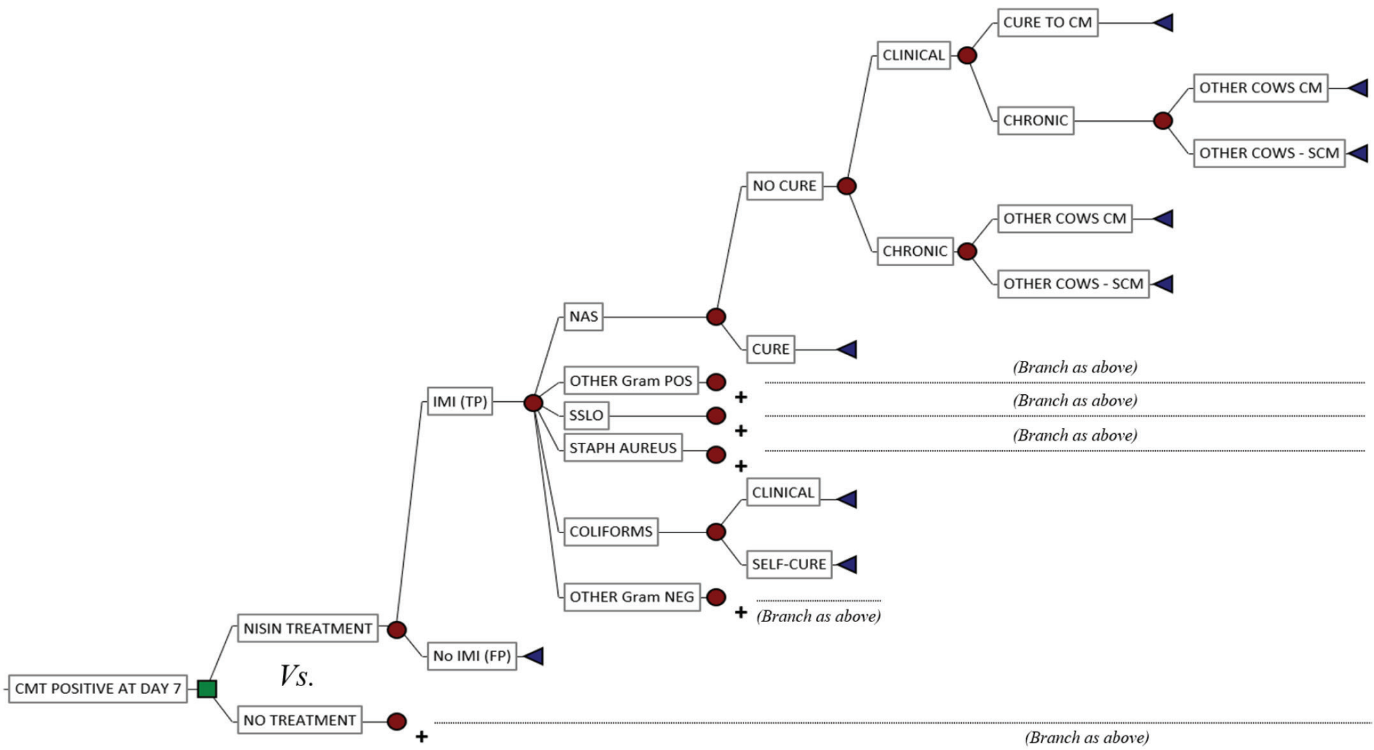

Figure 1. Schematic representation of the pathogen-specific IMI dynamics in the stochastic simulation model on the evaluation of the cost-benefit of subclinical mastitis treatment using nisin. Square represents a decision node, circles represent probability nodes, triangles represent end nodes, and plus signs indicate collapsed branches with an identical structure to the branch above.

mated as the expected spontaneous cure rates previously reported plus the marginal difference in bacteriological cure between quarters that received nisin and quarters that were not treated based on efficacy trials conducted as part of the FDA drug approval process (ImmuCell, Portland, ME; data shared with researchers). When SCM was left untreated, pathogen-specific spontaneous bacteriological cure rates were employed.

For pathogens without any documented efficacy of the use of nisin (i.e., *Staphylococcus aureus*) and quarters infected with gram-negative bacteria (which are intrinsically resistant), bacteriological cure after nisin treatment was equal to the spontaneous bacteriological cure rate for those pathogens (i.e., no treatment efficacy). All pathogen-specific cure rates were reported in Supplemental Table S1 (see Notes).

When bacteriological cure did not occur, quarters infected with gram-positive bacteria remained subclinically infected until the end of the lactation unless they became clinical. The probability and timing of clinical cases subsequent to SCM cases (occurring between 4 and 10 DIM) were etiology dependent. Cases of SCM caused by a gram-negative pathogens could result in spontaneous cure (with or without treatment) or become clinical, but did not become chronic. Clinical cases were assumed to receive IMM antibiotic treatment. Pathogen-specific probabilities of bacteriological cure after treatment of CM were used in the model. The probability of bacteriological cure of CM cases for multiparous cows was estimated to be 5% lower than bacteriological cure of primiparous cows (Pinzón-Sánchez and Ruegg, 2011).

Cows that remained chronically infected could potentially infect other cows which were assumed to develop SCM or CM. The number of cows that can develop secondary infections was estimated based on pathogen-specific probabilities as reported by Dalen et al. (2019b).

Economic Assumptions

The economic consequences of SCM included costs associated with CMT, SCM treatment, reduced milk yield, increased risk of culling, reduced reproductive performance, clinical cases, and transmission to other cows (Supplemental Table S2, see Notes).

Cost of CMT and SCM Treatment. The cost of CMT (reagent and labor) was \$0.28 per cow (minimum = \$0.15, maximum = \$0.39; Table 2). When no treatment protocol was followed, the cost of CMT was not consid-

Table 2. Baseline economic values used in the pathogen-specific stochastic model

Variable	Distribution	Values[1]	Source
Milk value[2] ($/kg)	Fixed	0.44	USDA NASS, 2023
Slaughter value ($/kg)	Fixed	1.98	USDA NASS, 2023
Replacement heifer cost ($)	Fixed	1,762	USDA NASS, 2023
Hourly wage ($)	Pert	14.5, 16.6, 17.1	USDA NASS, 2023
Labor for CMT (min/cow)	Normal-left truncated	1.5, 0.5, 0.5	Author's expertise
Cost CMT detergent ($/L)	Fixed	$4.33	Supplier
Reagent used in CMT (test/cow)	Fixed	20.0 mL	Industry supplier
Antibiotic treatment period of CM cases[3] (d)	Pert	3, 5, 8	Leite de Campos et al., 2021
Milk withhold period after treatment (d)	Fixed	3	Leite de Campos et al., 2021
Labor dedicated to CM treatment (min/cow/d)	Pert	5, 10, 15	Author's expertise
Cost of CM treatment ($/tube/cow)	Fixed	4.72	Leite de Campos et al., 2023
Labor dedicated to SCM treatment (min/cow/d)	Pert	0.5, 1.0, 1.5	Author's expertise
Cost of SCM treatment with nisin[4] ($/treatment)	Normal-truncated	30.0, 1.0, 29.0, 31.0	Author's expertise

[1]Values are expressed based on the following distributions: pert (minimum, mode, maximum), fixed (predetermined value with no variation), normal-left truncated (minimum, mean, maximum), or normal-truncated (mean, SD, minimum, maximum).

[2]Based on the monthly milk value between May 2018 and May 2023 as reported by the USDA.

[3]Number of days in antibiotic treatment. Milk from treated cows is discarded.

[4]The cost of SCM treatment with nisin includes intramammary infusions of nisin in the SCM infected quarter on 3 occasions at 24-h intervals (3 doses per treatment).

ered because there is no reason to conduct a CMT without further actions. The cost of SCM treatment included IMM treatments and labor (Table 2). Because the final cost of the IMM nisin product is unknown, a total cost of $30.00 ($1.00 SD; range $29.00–$31.00) was allocated for the treatment involving the 3 IMM tubes.

Reduced Milk Yield. Based on Halasa et al. (2009), reduced milk yield was estimated by parity and pathogen group after pathogen-specific increases in SCC and duration of SCM were specified. Resulting milk losses were proportionally adjusting based on the expected milk yield of the cows in our study. When cows achieved bacteriological cure, the cost of reduced milk yield was calculated for the period between the onset of SCM and the end of treatment, or until bacteriological cure for cows that cured spontaneously. Reduced milk yield for cows with chronic SCM was estimated for the period between the onset of SCM and the end of lactation (Table 1).

Cost of Clinical Cases. Cows with SCM that become clinical incurred both direct costs (drug, labor, and milk discard associated with treatment) and indirect costs (reduced milk yield after treatment, culling, mortality, and transmission). No costs were included for veterinary labor because treatments of mild and moderate cases of CM (which are the majority of cases) are routinely performed by farm personnel (Pinzón-Sánchez and Ruegg, 2011). Clinical cases were assumed to receive antibiotic treatment consisting of the administration of 200 mg/mL of IMM ceftiofur with a cost of $4.72 per day for an average of 5 d, with an additional 3 d of milk withholding (Table 2). This price was based on the actual price that 36 farmers paid for each tube of ceftiofur (Spectramast LC, Zoetis) after discount negotiations with suppliers as reported by Leite de Campos et al. (2023) and after adjustment for inflation. The amount of milk discarded was dependent on parity and DIM based on lactating curves (Table 1). Probabilities of achieving bacteriological cure for CM treated with ceftiofur (a broad-spectrum intramammary antibiotic) were dependent on the specific pathogen causing the infection.

Parity and pathogen-specific reductions in milk yield after cured CM events were estimated by developing quadratic regression equations (R statistical software; https://www.r-project.org/; Supplemental Table S3, see Notes) based on weekly milk losses reported by Gröhn et al. (2004).

Cows that remained infected after treatment and became chronic incurred additional costs related to reduced milk yield, reduced fertility at first breeding, pregnancy loss, and culling.

Reduced Reproductive Performance. Costs of reduced reproductive performance, as well as increased risk of culling and mortality attributable to SCM, were calculated using a Markov chain model developed by Cabrera (2012; https://dairymgt.cals.wisc.edu/tools/cow_value_resp_v2/) and added to our model. The cost of pregnancy failure at the first breeding was calculated as the difference in retention payoff of 2 average cows that are categorized similarly but differ in that one cow is not pregnant and the other cow is pregnant (De Vries, 2006). This calculation was estimated separately for primiparous and multiparous cows. First breeding was assumed to happen at 90 DIM. The probability of pregnancy at the first breeding was set at 39% and 32% for cows experiencing SCM and CM at the time of first breeding, respectively, and 41% for healthy cows (Fuenzalida et al., 2015).

The cost of pregnancy loss was calculated as the difference in retention payoff between similar cows with the difference of one of them having pregnancy loss, for pregnant primiparous and multiparous cows (De Vries, 2006). As pregnancy loss can occur at any time, the risk period was set to be between 90 DIM and the end of the lactation. Various scenarios with different levels of reduced milk yield and parity were calculated. The incidence of pregnancy loss was set at 9.7% and 5.8% for cows experiencing CM after pregnancy diagnosis and healthy cows, respectively (Santos et al., 2004).

Cost of Replacement. Replacement was calculated in 2 steps. First, deterministic values were obtained for each combination of reason (culling or mortality), cause (SCM or CM event), parity (primiparous or multiparous cows), and stage of lactation (early, mid, or late lactation) using Cabrera's model (https://dairymgt.cals.wisc.edu/tools/cow_value_resp_v2/). Briefly, the model calculated the difference between the future economic value of a cow and her potential replacement. The value of a cow is greater early in the lactation and decreases as time goes by. This value would continue a decreasing trend depending on pregnancy status, parity, and stage of lactation at the CM event. Second, a stochastic value was created for each combination of reason, cause, and parity using the 3 stages of lactation as the minimum, most likely, and maximum values in pert distributions.

The probability of culling after CM varied depending on the moment of culling in the lactation period and ranged between 3% to 6% for primiparous cows and 7% to 11% for multiparous cows (Hertl et al., 2011). The probability of culling SCM cows was assumed to be half of what was estimated for CM events (Hertl et al., 2011). Genetic improvement of replacement was set at 1.0%. Expected milk reduction from CM resolution until end of the lactation ranged from 0.11% to 0.9% in primiparous cows and from 0.19% to 1.47% in multiparous cows depending the moment of IMI (Seegers et al., 2003).

The cost of mortality was calculated only for CM because it is assumed that SCM does not result in mortality. The probability of mortality due to a CM event was between 0.9% and 5% for primiparous cows and 2% and 6% for multiparous cows varying depending on the onset of CM in the lactation (Hertl et al., 2011). Unlike culling, mortality did not include slaughter revenue.

Costs of Transmission. The costs of subsequent SCM cases after transmission were estimated by multiplying the cost of the original case by the number of additional cases resulting from transmission (Down et al., 2013). The total cost of SCM was estimated by totaling the costs from the original and secondary cases, following transmission. All costs were calculated per pathogen group and parity and are a weighted average according to the probability of the different subsequent events that each cow may experience.

Sensitivity Analysis

Sensitivity analyses were conducted to assess the economic impact of using nisin treatment under an array of potential values for the most meaningful input variables, as well as to determine the break-even point at which nisin treatment would have no economic impact on SCM treatment. During the sensitivity analysis, only the evaluated variable was changed, and all other variables remained unchanged. The sensitivity analysis was performed for marginal cure rates with nisin treatment (using 5% increase steps from the baseline), cost of nisin treatment (range from $20 to $50 per 3 d of treatment), value of milk (range between May 2018 and May 2023), variations in test characteristics of CMT, and different parity structures within herds (using 2% steps from the baseline).

RESULTS

Net Cost of Subclinical and Clinical Mastitis Cases Per Pathogen During Lactation

The estimated cost of subclinical intramammary infection (only true-positive cases) occurring during early lactation was on average $170 per case, ranging between $148 and $187 (90% range). The cost varied depending on the etiology and parity (Table 3). The cost of subclinical intramammary infection was less when caused by NAS, which was estimated at $122 (90% range = $101–$148) for primiparous cows and $193 (90% range = $163–$221) for multiparous cows both including the costs associated with transmissions. The most expensive case of subclinical intramammary infection was caused by *Staphylococcus aureus*. The costs of subclinical intramammary infection also include the costs associated with the probability of the cows developing CM.

If a subclinical case progressed to CM, the cost of the CM case occurring at any given point during the lactation was estimated at an average of $521 (90% range = $435–$581), estimated at $374 (90% range = $313–$432) for primiparous cows and $587 (90% range = $495–$681) for multiparous cows both including the costs associated with transmissions.

Economic Impact of SCM Treatment Using Nisin

The estimated cost of SCM restricted to cases occurring at 7 DIM, identified using CMT, (which include true- and false-positive cases), averaged $165 per case per lactation (90% range = $138–$194) under no treatment protocol. Because the CMT is an imperfect test, the false-positive cases were 19% (90% range = 11%–26%), whereas the true-positive cases were 81%

Table 3. Cost of subclinical intramammary infection in early lactation by pathogen group and parity per lactation, accounting for transmission to other cows in the herd

Pathogen[1]	Primiparous cows only, $ per case (90% range)	Multiparous cows only, $ per case, (90% range)	Average herd structure,[2] $ per case (90% range)
Including transmission			
Staphylococcus aureus	520 (441, 615)	922 (773, 1,081)	767 (635, 922)
NAS	122 (101, 148)	193 (163, 221)	165 (130, 198)
SSLO	208 (161, 254)	386 (311, 484)	321 (237, 424)
Other gram-positives	93 (72, 115)	144 (125, 174)	126 (97, 157)
Coliforms	30 (23, 38)	47 (40, 53)	41 (34, 47)
Other gram-negatives	31 (25, 35)	55 (43, 67)	46 (36, 55)
All pathogens[3]	121 (102, 142)	196 (163, 221)	170 (148, 187)
Without transmission			
Staphylococcus aureus	134 (117 151)	239 (201, 269)	198 (174, 227)
NAS	44 (36, 52)	70 (58, 82)	60 (50, 71)
SSLO	74 (57, 94)	139 (102, 176)	115 (87, 147)
Other gram-positives	50 (39, 60)	76 (60, 92)	67 (52, 80)
Coliforms	30 (24, 37)	48 (39, 53)	41 (34, 47)
Other gram-negatives	30 (23, 37)	56 (42, 68)	46 (36, 56)
All pathogens[3]	50 (43, 57)	82 (68, 93)	70 (59, 80)

[1]Other gram-positives = *Actinomyces* spp., *Bacillus* spp., *Corynebacterium* spp.; coliforms = *Escherichia coli, Klebsiella pneumoniae*; other gram-negatives = *Pseudomonas* spp., *Serratia* spp.

[2]Average herd structure based on replacement rate (37% most likely, ranging from 32% to 44%).

[3]All pathogens refers to the weighted average cost of subclinical IMI ($/case) by pathogen. For instance, the average cost of subclinical IMI in early lactation is $121 among primiparous cows and $196 for multiparous cows (including transmission). For an average herd, the average cost of subclinical IMI in early lactation is $170.

(90% range = 74%–89%). The cost of SCM identified at d 7 using CMT and treated using IMM nisin was estimated to average $145 per case per lactation (90% range = $122–$174). Hence, when comparing treatment protocols, use of IMM nisin to treat SCM during the first week of lactation resulted in an average economic benefit estimated at $19 per treated case (Figure 2). Given the 90% range around the average economic impact, each CMT-positive case treated with nisin could generate a saving of up to $42, but also extra costs of up to $1.80 depending on the characteristics of the herd. Assuming a 1,000-cow herd, this variation represents up to $12,350 in savings and up to $475 in extra costs per year if nisin is used according to this protocol. A positive economic impact was observed in 93% of the simulated herds.

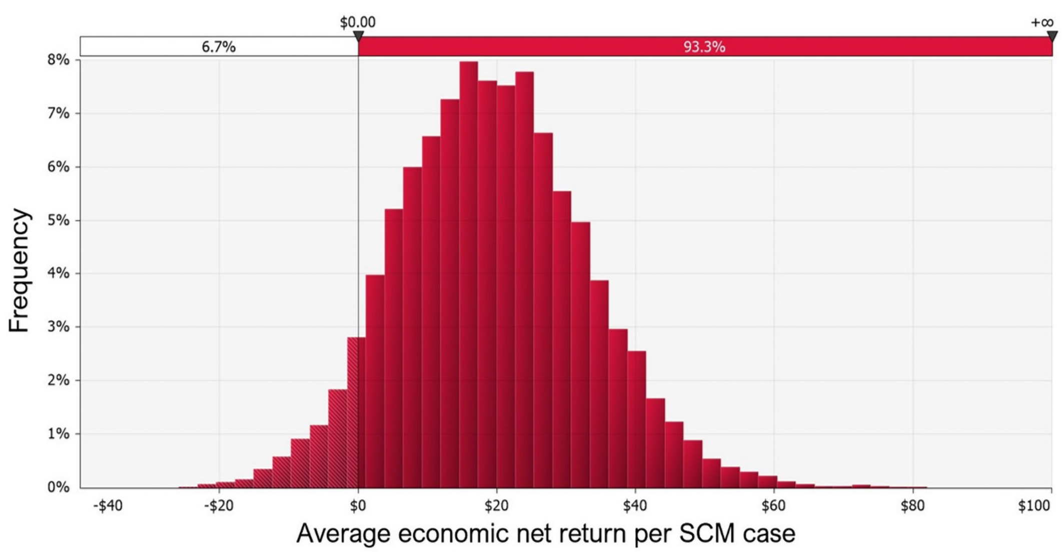

Figure 2. Graphic representation of the average economic net return of subclinical mastitis treatment at 7 DIM using nisin-based intramammary tubes, expressed in USD/case run 10,000 iterations. The average economic net return was $19.46/SCM case (90% range = −$1.80 to $42.17). Savings were observed in 93.3% of the iterations. This indicates that implementing an SCM treatment protocol using nisin-based IMM tubes in cows diagnosed with CMT at 7 DIM could generate an average savings of $19.46 per cow.

Sensitivity Analysis

The marginal bacteriological cure rate of nisin treatment (based on the spontaneous bacteriological cure rate) was the factor that most influenced the model, contributing 73% to the variability of the economic benefit. All other factors had less than 5% influence on the variance of the result and included factors such as specificity of CMT, days of CM treatment, days in milk at CM event, sensitivity of CMT, lactation length, transmission rates, prevalence of IMI, and SCC due to IMI.

The marginal bacteriological cure rate represents the additional cure rate of SCM cases obtained by using nisin, as compared with the spontaneous cure rate associated with lack of treatment. To generate economic savings in most treated SCM cases, the nisin protocol needs to generate an average marginal cure rate of at least 27% over the spontaneous cure rate (Table 4). This value is defined as the break-even point when the economic benefit per CMT-positive cow treated with nisin in a random herd would be equal to zero, and considering the probability distribution around the economic impact, half of the herds would generate savings and the other half would generate losses. Assuming all other variables remained constant, an average marginal cure rate under the break-even point would generate economic losses, and a rate over the break-even point would generate savings. The break-even point of marginal cure rate differed by parity, as primiparous cows (i.e., first lactation cows) had a break-even point of 33%, and multiparous cows (i.e., second or greater parity) had a break-even point of 24%.

The cost of nisin treatment was another influential factor in the economic impact. The break-even point of the nisin treatment cost was $49 on average (Figure 3), with a value of $37 for primiparous cows and $57 for multiparous cows. This means that greater treatment costs would still be economically beneficial in multiparous compared with primiparous cows. Moreover, increasing the cost of nisin treatment by $5 over the assumed $30 cost used in the model would reduce the savings to $14 per case (90% range = −$8 to $39). Variations in milk value had influenced the economic impact of the nisin protocol (Figure 3). The break-even point of milk value was estimated at $0.22 per kg ($10/cwt). Therefore, the nisin protocol would not be economically beneficial for the average herd when the milk value is under this value. The break-even point was $0.33/kg ($14/cwt) for primiparous and $0.18/kg ($8/cwt) for multiparous cows. A 20% milk value decrease from the baseline (from $0.44/kg to $0.35/kg) still resulted in a positive economic impact (i.e., saving) of $11 per treated cow.

Based on the CMT test characteristics, the sensitivity analysis indicated that with an increase in specificity from 83.9% to 95% and a decrease in sensitivity from 71% to 50%, the net economic impact of using nisin would increase from $19 to $25 per SCM case and was economically advantageous in 95.2% of the iterations

Table 4. Sensitivity analysis of the total costs of subclinical mastitis at 7 DIM under different treatment decisions, net economic impact, and cost-benefit based on various marginal cure rates between spontaneous cure and nisin treatment

Marginal cure rate with nisin treatment,[1] %	With nisin treatment,[2] $ per case (90% range)	No treatment,[2] $ per case (90% range)	Net economic impact,[3] $ per case (90% range)	Cost-benefit, %[4]
Equal[5]	203 (172, 243)	165 (133, 202)	−38.5 (−53.7, −24.1)	0.0
+5	196 (166, 231)	165 (133, 200)	−31.2 (−47.0, −17.3)	0.0
+10	189 (162, 220)	165 (135, 200)	−23.9 (−38.4, −10.1)	0.6
+15	182 (155, 211)	165 (134, 198)	−16.5 (−30.5, −3.5)	1.8
+20	174 (147, 205)	165 (132, 200)	−9.2 (−23.1, 4.4)	13.6
+25	167 (141, 193)	165 (132, 199)	−1.8 (−15.0, 12.0)	61.4
+30	160 (136, 184)	165 (134, 201)	5.3 (−8.88, 20.4)	72.4
+35	152 (131, 177)	165 (134, 198)	12.3 (−1.0, 27.7)	91.8
+40	145 (124, 169)	165 (132, 202)	20.1 (5.4, 35.4)	98.8

[1]Marginal bacteriological cure rates (additional bacteriological cure rates obtained from applying treatment) = (nisin bacteriological cure rates) − (spontaneous bacteriological cure rates). Spontaneous cure rates (means): NAS = 44.9%; SSLO = 39.3%; other gram-positive = 53.7%.

[2]Values represent the most likely cost of a SCM case (90% range) for the given treatment decision under the assumption in the first column.

[3]Net economic impact (Δ Cost between treatments) = (cost of SCM case if no treatment) − (cost of SCM case if nisin treatment). Positive values represent positive net profits and negative values represent economic losses.

[4]Values represent the percentage of simulations in which the treatment with nisin is economically beneficial compared with the no treatment decision. For instance, a cost-benefit of 50% indicates equal probability that the nisin treatment generates savings than losses; thus, the net economic impact would equal $0.00 (break-even point).

[5]Equal means no difference in bacteriological cure rates between nisin treatment and no treatment (i.e., zero treatment efficacy).

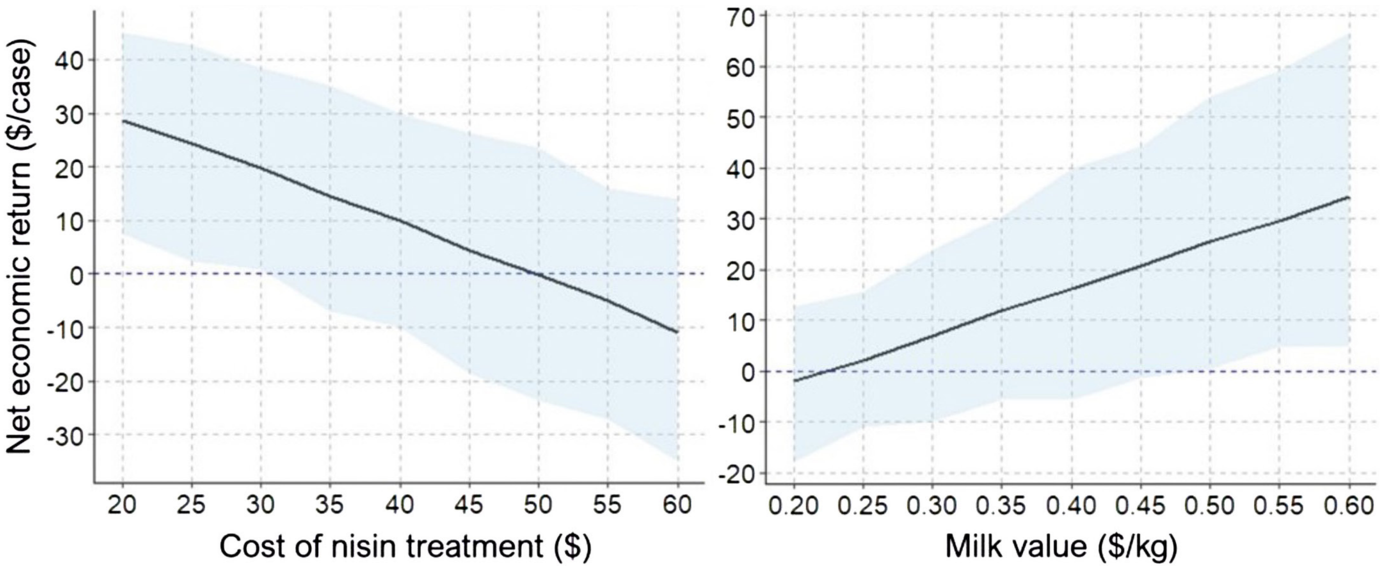

Figure 3. Sensitivity analysis of the net economic return ($/case, 5th and 95th percentiles in shaded area) for treating subclinical mastitis with nisin at 7 DIM under various nisin treatment costs (assuming 3 IMM doses; left side) and milk values (right side). When the nisin treatment cost was below $49 or milk value was over $0.22 per kg ($10/cwt), SCM treatment with nisin was profitable, with over 50% of the iterations resulting in a net economic return of $0.00 or higher.

thanks to the reduction of false-positive treated cases from 19% to 10%. At the herd level, as the specificity increased, more herds would benefit economically from treatment. In terms of the parity structure of the herds, as the proportion of multiparous cows increases, the probability of generating savings by implementing the nisin treatment increases (Table 5). On the other side, as more primiparous are in the herd, the probability of economic success reduces. We found a probability of 95% of generating savings when nisin treatment was administered only to multiparous cows, generating an average net economic impact of $27 per SCM case compared with

Table 5. Sensitivity analysis of the total costs of subclinical mastitis at 7 DIM under different treatment decisions, net economic impact, and cost-benefit at various levels of sensitivity and specificity of CMT and parity structure

Item	With nisin treatment,[1] $ per case (90% range)	No treatment,[1] $ per case (90% range)	Net economic impact[2] $ per case (90% range)	Cost-benefit[3]
CMT test characteristics				
95% Se, 71% Sp	139 (116, 163)	155 (131, 184)	16.4 (−4.6, 37.8)	90.2%
71% Se, 84% Sp	146 (121, 172)	165 (138, 194)	19.6 (−1.1, 40.8)	93.0%
50% Se, 95% Sp	159 (132, 189)	184 (153, 216)	25.2 (0.1, 51.3)	95.2%
Parity structure (first/second or greater parity, %)				
100% first parity	114 (96, 134)	121 (99, 146)	7.1 (−8.9, 24.6)	73.8%
34%/66%	147 (121, 173)	167 (138, 199)	20.2 (−2.6, 45.4)	93.4%
36%/64%	146 (120, 176)	166 (136, 202)	19.9 (−2.4, 44.9)	92.4%
38%/62%	145 (121, 174)	165 (137, 197)	19.3 (−1.6, 41.8)	91.6%
40%/60%	144 (120, 172)	163 (131, 197)	19.0 (−2.5, 42.5)	91.5%
42%/58%	143 (119, 171)	162 (132, 195)	18.9 (−1.6, 41.5)	90.6%
100% ≥second parity	164 (134, 198)	191 (154, 230)	26.9 (0.65, 53.5)	95.2%

[1]Values represent the most likely cost of an SCM case (90% range) for the given treatment decision under the assumption in the first column.

[2]Net economic impact (Δ cost between treatments) = (cost of SCM case if no treatment) − (cost of SCM case if nisin treatment). Positive values represent positive net profits, and negative values represent economic losses.

[3]Values represent the percentage of simulations in which the treatment with nisin is economically beneficial compared with the no treatment decision. For instance, a cost-benefit of 50% means that there is equal probability that the nisin treatment generates savings than losses; thus the net economic impact would equal $0.00 (break-even point).

allocating the treatment only among primiparous cows, which generated savings in 73% of the SCM cases, averaging $7 per SCM case.

DISCUSSION

Our pathogen-specific stochastic model suggests that IMM treatment of SCM using nisin in cows diagnosed using CMT one week after calving is economically profitable in most herds. Under default conditions, the treatment using nisin reduced costs of SCM as compared with no treatment, thus generating a positive economic impact. However, the economic success of treatment is highly dependent on the accurate identification of IMI cases. It is important to notice the distinction between SCM (defined based on SCC response as detected by CMT) and IMI (active bacterial infection that is culture positive). Although treatments would be ideally administered to cows with IMI, SCM defined based on increased SCC using CMT serves as a cheaper and more practical alternative to bacteriology. In our model, 18.7% of the cows diagnosed as having SCM based on CMT were false-positive cases. By exclusively treating true-positive cases with nisin, the average net economic impact would have amounted to $31 per case. Therefore, this case definition represents a disadvantage for any treatment intervention of SCM given the potential unnecessary treatment of false-positive cases which reduces the profits. A way to reduce the number of false-positive cases is by increasing the specificity of the CMT, which was economically advantageous. Increased specificity may be achieved by diagnosing SCM based on CMT results equal to or greater than score +1 (rather than on trace) but would leave some IMI without treatment affecting the economic benefit of the intervention at the herd level. Conversely, the benefit of increasing the sensitivity did not outweigh the extra false-positive cases caused by the decreased specificity. Other alternative options exist to increase the specificity such as using CMT and bacteriological culture in series to detect IMI, or the use of DHI test(s) or Fourier-transform infrared spectroscopy to help making the treatment decision. However, the costs associated with bacteriological tests or Fourier-transform infrared spectroscopy in addition to false negative cases may easily exceed potential economic benefits. The use of one or multiple DHI tests to guide treatments is a lower cost alternative. However, the treatment would be administered at the time of DHI results, which may differ from early lactation and would likely result in a lower sensitivity of detection and effectiveness of treatments. Although they are not perfect, these alternatives could be evaluated in future research.

Most economic evaluations of mastitis focus on CM. Our estimates were consistent with the costs of CM cases previously reported ranging from $338 to $594 per case (Sørensen et al., 2010; Pinzón-Sánchez et al., 2011; Heikkilä et al., 2012; van Soest et al., 2016; Doehring and Sundrum, 2019). Model assumptions, including the selection of input variables, strongly influence the variability among previously reported estimates (Hogeveen et al., 2011; Raboisson et al., 2020). For instance, in addition to the direct cost of mastitis (drugs and milk discard associated with the treatment), some researchers have included the impact of mastitis in milking production after clinical resolution, as well as the disease spread (van den Borne et al., 2010; Cha et al., 2013; Doehring and Sundrum, 2019). Most models have focused on the assessment of protocols for prevention and control of a specific pathogen (Swinkels et al., 2005; Steeneveld et al., 2007) whereas others focused on the most influential pathogens in a representative herd (Sørensen et al., 2010; Cha et al., 2011).

The pathogen-specific transmission rate had a different impact on our model depending on the presentation of mastitis (SCM as compared with CM cases). Although the transmission rate contributed 53% of the overall cost of SCM cases, it represented only 11% of the total costs of CM cases. This is attributable to the prolonged contagiousness of untreated chronic subclinically infected cows. Similarly to our result, Bonestroo et al. (2023) reported that the transmission of contagious bacteria (responsible for most SCM cases) accounted for 45% of the total cost of chronic mastitis. The pathogen-specific transmission rate of contagious bacteria is expected to vary among pathogens and mastitis management (Exel et al., 2022). Given the limited quantitative knowledge of pathogen-specific transmission rate, the variation in the transmission rate was accounted for in our model by including a 10% variability around estimates by Dalen et al. (2019a) as previously approached by Bonestroo et al. (2023).

Sensitivity analyses revealed that parity structure influenced the net economic impact of treatment of SCM using nisin. Although the nisin treatment in early lactation was economically beneficial for herds with typical parity structures, the economic benefit was greater among multiparous cows as compared with primiparous cows. The different economic benefit between primiparous and multiparous cows involved a combination of parity-specific factors such as milk yield, bacteriological cure rates, SCC at treatment, milk losses due to treatment, milk drop after a mastitis event, culling, mortality, reproductive failure, and pregnancy losses, which accounted for positive or negative contributions to the different economic impact by parity (Sol et al., 1997; Gröhn et al., 2004; Gonçalves et al., 2018). However, the primary driver of the difference was the level of production. Every event affecting milk productivity, such as losses due to treatment and milk reduction, had a more significant negative effect on multiparous cows due to their higher productivity. For instance, the likelihood of bacteriological cure of cows with SCM has been reported to be 65% lower in cows with ≥3 lactations compared with lower lactation cows (Sol et al., 1997). Still, because multiparous cows have greater milk yield, prevention of SCM cases evolving into clinical by using nisin can offset reduced rates of BC and enhance the economic benefits of the treatment. As a practical consideration, producers may prefer to target multiparous cows for nisin treatment, thus enhancing the likelihood and magnitude of economic net benefits.

Bacteriological cure rates had a major impact on the variability of the average net economic impact. This occurred because of the wide distributions based on uncertainty about pathogen-specific BC for cows with SCM treated with nisin. During the efficacy trials as part of the FDA approval process, the observed spontaneous BC rates were less than rates previously reported (Supplemental Table S1, see Notes). Consequently, we calculated the marginal difference in cure rates between the use of nisin and the negative control groups. By adopting this approach, we were able to generate cost estimates that align with the cure rates reported in the literature along with preventing any overestimation of potential benefits associated with nisin treatment. Another advantage of using marginal values is that the benefit of using nisin is relative to the spontaneous bacteriological cure achievable depending on each pathogen (Ruegg, 2021).

We defined value of milk and parity within narrow ranges because they are unlikely to vary substantially within a year and among farms, as shown in the most recent USDA database (USDA ERS, 2023) with a reported average interannual variation of $0.15/kg ($7.00/cwt) between May 2018 and May 2023. Because it is reasonable that variation in milk value and cost of nisin treatment may have an impact on the result, both were explored in the sensitivity analysis. The exact price of treatment using nisin is unknown because the product is not yet commercially available, and this limitation was addressed by choosing a conservative price estimate (at least twice the price of the average intramammary antibiotics that are currently marketed). Moreover, limited treatment cost uncertainty was included to reflect the slight variation that farms encounter during their negotiations for treatment drugs. The break-even point of the treatment cost reported for the average cow, as well as primiparous and multiparous cows, serves to evaluate different prices on the market as compared with the $30 used in our model.

Although milk value is a well-known factor with a great influence on the cost of CM (Rollin et al., 2015), it was not very influential in our model. This is likely because neither treatment using nisin nor failure to treat SCM requires milk discard. Most of the influence of milk value in our model is driven by the cost of SCM cases that become clinical and are subsequently treated with products that require milk discard. The break-even point for the milk value was $0.22/kg ($10/cwt), which is close to the lowest US milk value recorded during the period between 2018 and 2023 and is under the production cost in the United States (USDA ERS, 2023). The use of waste milk containing antibiotic residues may alleviate some costs associated with CM and thus would be expected to reduce profitability of the treatment strategy with nisin. However, this practice carries risks related to calves' health and performance and can potentially contribute to the development of antimicrobial resistance (Aust et al., 2013; Firth et al., 2021). It is worth noting that although we approached the sensitivity analysis modifying one variable at the time to determine the impact of the variable in the entire model, endless interactions between variables are expected. Factors such as milk value, herd parity structure, and cure rates (which depend on prevalence of pathogens and strains) are constantly changing and playing a role in the economic impact of the treatment. Thus, although understanding the importance of each variable in the model serves to make sound decisions, their interactions need to be kept in mind.

CONCLUSIONS

Based on our pathogen-specific stochastic simulation model, treatment of cows diagnosed with subclinical mastitis at 7 DIM using nisin-based IMM tubes is expected to generate positive economic benefits and was the optimal economic strategy for most herds. The economic benefit of treating SCM using IMM nisin varied according to factors such as bacteriological cure rates, parity, or the cost of nisin. The uncertainty related to nisin efficacy and cost was accounted for in the model. A break-even point was observed when the average bacteriological cure rate with nisin was 27% greater than the spontaneous cure rate, when the cost of nisin treatment was $49, and when milk value was $0.22/kg ($10/cwt). Our model results suggest that to maximize the probability of positive economic impact of nisin treatment, dairy producers may target multiparous cows during periods when milk value is high.

NOTES

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Abbreviations used: CM = clinical mastitis; CMT = California Mastitis Test; FDA = Food and Drug Administration; Gram POS = gram-positive bacteria; Gram NEG = gram-negative bacteria; IMM = intramammary; SCM = subclinical mastitis; Se = sensitivity; Sp = specificity; SSLO = *Streptococcus* spp. and *Streptococcus*-like organisms; TP = true positive.

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