Ciência Rural, Santa Maria, v.52:11, e20210505, 2022

http://doi.org/10.1590/0103-8478cr20210505

ISSNe 1678-4596

MICROBIOLOGY

Farm microbiological milk culture: study case on cow performance, financial and economic aspects

Nathan Felipe Fontoura Reis[1] **Fernanda Carolina Ferreira**[2] **Hilton do Carmo Diniz Neto**[1] **Hemilly Cristina Menezes de Sá**[1] **Sandra Gesteira Coelho**[1*]

[1]Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), 30161-970, Belo Horizonte, MG, Brasil. E-mail: sandragesteiracoelho@gmail.com.[*]Corresponding author.

[2]Veterinary Medicine Teaching and Research Center, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Tulare, California, United States of América.

ABSTRACT: *This study evaluated the use of microbiological culture of milk from cows with clinical mastitis (CM), and the performance and economic results after implementing this procedure. The 18-month data were obtained from a farm in Minas Gerais State, Brazil, with an average daily production of 23.1 L of milk from cows milked twice daily under a semi-intensive regime. After a case of CM was identified, a milk sample from the affected quarter was collected and sent to the farm's laboratory. First, a bi-plate containing selective growth medium was used for isolation of Gram-positive and negative bacteria (Plate 1). Subsequently, a tri-plate with selective growth medium was used for isolation of Gram-positive and negative bacteria, and bacteria of the genus Streptococcus (Plate 2). Finally, a tri-plate containing three chromogenic culture media capable of identifying 18 bacterial species was used (Plate 3). Clinical cases of mastitis were treated once a day based on the results of the microbiological culture. Two economic scenarios were evaluated (scenarios 1 and 2). Scenario 1 compared the situation if all cases of CM were treated (not using on-farm culture) vs. the use of on-farm culture (real data) and the generated savings for one year. Data from 1,582 lactations of 1,227 cows were evaluated, with 1,917 cases of CM from 636 cows recorded. The average annual incidence of CM was 48.2%. Of all cases evaluated, 76.8% were classified as grade 1 mastitis; 20% as grade 2, and 3.2% as grade 3. The incidence of new clinical cases of mastitis was 4.17% per month. From the samples analyzed on the three plates, 27.8% of the cases received a recommendation to not be treated and 72.2% received a recommendation of treatment. However, only 18.6% were not treated, making a total of 81.4% treated cases. Of the clinical cases that did not receive intramammary antibiotic therapy, 84.3% had a clinical cure. Conversely, the clinical cure rate reached 84% for the cases that received intramammary antibiotic therapy. In scenario 1, the total operational cost of the clinical case decreased by 10.3% after the implementation of the on-farm culture, with an 18.4% reduction in the use of antibiotics. In scenario 2, there was a 5.5% reduction in the cost of the clinical case and an 11.8% reduction in the use of antibiotics. Thus, the implementation of on-farm culture and the applied methodology, enhanced treatment accuracy of CM cases, reducing the total operating cost of the case and the use of antibiotics on the farm.*

Key words: *cost, treatment, intramammary antibiotic therapy, plate.*

Cultivo microbiológico de leite na fazenda: estudo de caso sobre desempenho de vacas, aspectos financeiros e econômicos

RESUMO: *O objetivo deste estudo foi avaliar a utilização da cultura microbiológica de leite de vacas com mastite clínica (MC), os resultados zootécnicos e econômicos após implementação desse procedimento. Dados de 18 meses foram obtidos em uma fazenda em Minas Gerais com produção média de 23,1 L de leite/vaca/dia, em duas ordenhas diárias em regime semi-intensivo. Após a identificação da MC, uma amostra de leite do quarto afetado foi coletada e encaminhada para laboratório da fazenda. Primeiramente foi utilizada placa bipartida em meio de cultura seletivo para crescimento de bactérias Gram-positivas e negativas (Placa 1), seguindo-se para a utilização da placa tri-partida com meios de cultura seletivos para crescimento de bactérias Gram-positivas e negativas e bactérias do gênero Streptococcus (Placa 2) e posteriormente para placa tripartida, contendo três meios de cultura cromogênicos, capazes de identificar 18 espécies bacterianas (Placa 3). Foram avaliados dados de 1.227 vacas em 1.582 lactações, sendo registrados 1.917 casos de MC. Os casos clínicos de mastite foram tratados uma vez ao dia com base nos resultados da cultura microbiológica. Foram realizadas duas avaliações financeiras (cenário 1 e 2). O cenário 1 comparou a situação se todos os casos de MC fossem tratados (ausência de cultura na fazenda), comparado com a utilização da cultura na fazenda (dados reais) e as economias de recursos geradas durante o período de um ano. O cenário 2 utilizou os resultados reais após a economia de recursos gerados pela cultura, seguindo a recomendação ideal de tratamento. Foram avaliados 1.917 casos de MC (636 animais), totalizando incidência média anual de 48,2%. Do total dos casos avaliados, 76,8% foram classificados como mastite grau 1; 20% grau 2 e 3,2% grau 3. A incidência de novos casos clínicos de mastite foi de 4,17% ao mês. Das amostras analisadas nas três placas, 27,8% dos casos receberam a recomendação de não serem tratados e 72,2% de tratamento. Entretanto, apenas 18,6% realmente não foram tratados, totalizando 81,4% de tratamentos. Dos casos clínicos que não receberam antibioticoterapia intramamária, 84,3% apresentaram cura clínica. Já os casos que passaram por antibioticoterapia intramamária, a taxa de cura clínica foi de 84,0%. No primeiro cenário, após a implementação da cultura na fazenda, o custo operacional total do caso clínico reduziu em 10,3%, com redução de 18,4% de utilização de antibióticos. Já no segundo cenário, houve redução de 5,5% no custo do caso clínico e redução de 11,8% na utilização de antibióticos. A implementação da cultura na fazenda e a metodologia aplicada, promoveu maior assertividade dos tratamentos dos casos de MC, com redução do custo operacional total do caso e da utilização de antibióticos na propriedade.*

Palavras-chave: *custo, tratamento, antibioticoterapia intramamária, placa.*

Received 07.01.21 Approved 11.12.21 Returned by the author 04.13.22
CR-2021-0505.R2
Editors: Rudi Weiblen Juliana Felipetto Cargnelutti

INTRODUCTION

Mastitis is one of the main diseases that affect dairy cows, and it is responsible for huge losses in the dairy sector (POL & RUEGG, 2007). When diagnosing a clinical case, the most common treatment strategy is to use intramammary or systemic antibiotic therapy depending only on the severity of the case. However, there has been growing concern regarding the use of antimicrobials in animal production in recent years, mainly due to the resistance of bacteria to commonly used antibiotics (FISCHER et al., 2019) and the cost.

About 50% of antibiotics used on dairy farms come from treatments related to mastitis (LAGO et al., 2011a). At the same time, the use of antimicrobials can be reduced by the adoption of prevention strategies and knowledge about the distribution of pathogens associated with the occurrence of clinical mastitis. The development of diagnostic methods for mastitis has gained prominence and several tests have been developed in recent years. The principle of on-farm culture is rapid results that allow producers to make strategic treatment decisions for clinical cases at the farm (FERREIRA et al., 2018). These tests have become an upcoming diagnostic tool and decision criterion for identification and treatment of clinical mastitis cases in dairy cows (MANSION-DE VRIES et al., 2014). However, the wide variation in the sensitivity and specificity of the tests, and primarily the on-farm applicability, limits the use of many in the farms (CHAKRABORTY et al., 2019).

Microbiological diagnosis of milk in cases of clinical mastitis usually results in approximately one third of Gram-negative bacteria, one third of Gram-positive bacteria, one third without bacterial growth, and 10.0% of other pathogens (OLIVEIRA et al., 2015). Antimicrobial treatment is dispensable in some cases, such as in cases of mastitis caused by *E. coli* or cases without bacterial growth (PYÖRÄLÄ, 1994; OLIVEIRA & RUEGG, 2014). Thus, treatment based on the results of on-farm culture provides the opportunity to identify the agent associated with the clinical case and the possibility of reducing the use of antimicrobials (LAGO et al., 2011a, 2011b; VASQUEZ et al., 2017).

Most of the published researches evaluating the implantation of on-farm culture were carried out in the United States, in confined herds, with a low prevalence of contagious pathogens and under different climatic conditions, genetic, and production system diversity than those reported on Brazilian farms (LAGO et al., 2011a, 2011b; FUENZALIDA & RUEGG, 2019; FERREIRA et al., 2018). We believed this is the first study that evaluated the implantation of the on-farm culture under typical Brazilian conditions.

Therefore, study case documented the use of on-farm microbiological culture of milk from cows with clinical mastitis on cow health, and evaluated the economic opportunities of this strategy in a farm in southeast Brazil.

MATERIALS AND METHODS

Data from April 2018 to September 2019 were used, made available by a farm located in the midwestern part of the state of Minas Gerais, Brazil. The data were obtained from the herd management software (IDEAGRI Ltda, Belo Horizonte, Minas Gerais), following the guidelines of the Ethics Committee on Animal Use (CEUA) of the Universidade Federal de Minas Gerais (UFMG), under the protocol number 284/2019.

Experimental farm and management of animals

The herd consisted of crossbred animals (Holstein x Gyr and Holstein x Brown Swiss) raised in a semi-intensive system, with an average daily production of 23.1 L of milk. The animals were milked twice a day (05:00 and 16:00). During the wettest months (October to April), the animals were kept in rotational paddocks with Mombasa grass (*Panicum Maximum* cv. Mombasa). After each milking, the cows received supplementation with concentrate in the feeding lane. In the dry season (May to October), the cows were confined in paddocks where they received a total diet twice a day, composed of corn silage and concentrate according to the recommendations of the NRC (2001).

At the dry-off (60 days before the next expected calving date), all animals received intramammary antibiotic therapy with cloxacillin (Orbenin Extra Dry Cow, Zoetis, São Paulo, Brazil) and teat sealant (Teat Seal, Zoetis, São Paulo, Brazil) in all quarters. Moreover, vaccination with *E. coli* J5 (Rotatec J5, Biogénesis Bagó, São Paulo, Brazil) was performed on the dry-off day, 30 days after drying-off, and 50 days after calving.

Identification of the clinical case of mastitis and collection of milk sample for culture

Mastitis cases were classified according to the severity of the case (WENZ et al. 2001), based on the visual evaluation of the milk after forest ripping and the presence of symptoms in the animal.

After observing a clinical case, a milk sample from the quarter affected was obtained. The first three streams were discarded and teats were cleaned with pre-dipping solution, then teats were disinfected using 70% ethanol, and milk samples were collected in sterile 30 mL conical tubes. Immediately after collection, the samples were sent to the laboratory. The cow was then separated and identified in the forelegs and hind legs using a leg band. Data on the degree of severity of mastitis, date of clinical diagnosis, and affected quarter were recorded in the management system.

Microbiological diagnosis and treatment definition

The microbiological diagnosis of clinical cases of mastitis was implemented on the farm in March 2018. Initially, a bi-plate with Blood Agar and MacConkey culture medium was used (Plate 1), for identification of Gram-positive and Gram-negative bacteria, respectively (Semac Labmast, Rio Grande do Sul, Brazil; Figure 1). In june 2018, the tri-plate containing Agar blood, Edward and MacConkey culture medium began to be used (Plate 2), selective for the growth of Gram-positive bacteria, bacteria of the genus *Streptococcus* and Gram-negative bacteria, respectively were used (Semac Labmast, Rio Grande do Sul, Brazil; Figure 1). In may 2019, the tri-plate containing three Chromogenic culture media (Plate 3) (AccuMast, FERA Animal Health, New York, USA; Figure 1) began to be used. This plate allowed the identification of 18 species of bacteria that cause mastitis by the color of the different colonies.

The samples were inoculated in a specific medium and incubated at 37 ºC for 24 hours. After the microbiological diagnosis, the treatment recommendations (Figure 1) were passed on to

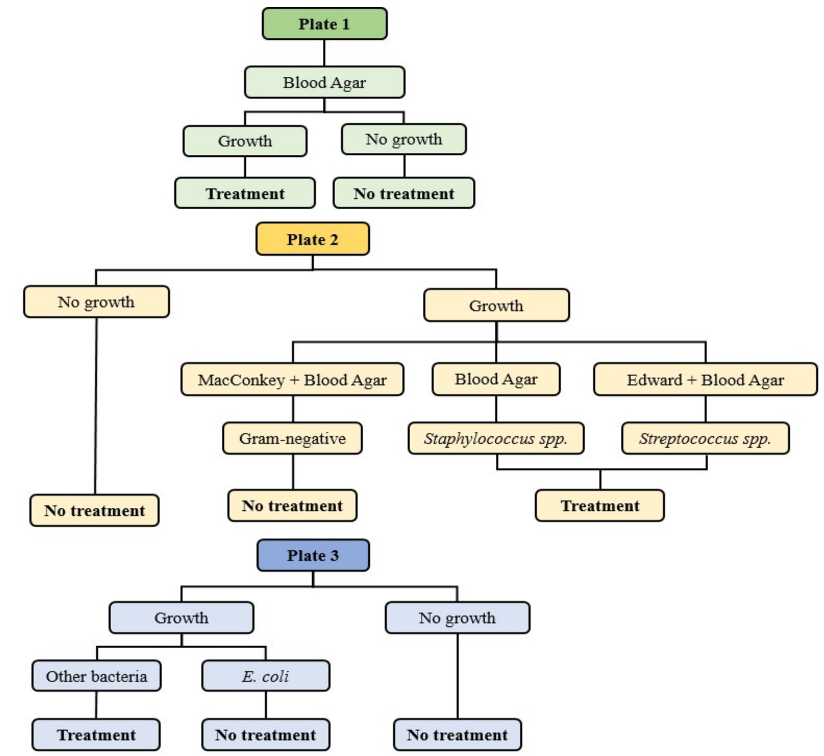

Figure 1 - Growth medium used during the microbiological diagnosis on the farm (Plate 1- Blood agar and MacConkey; plate 2- Blood agar, Edward and MacConkey and plate 3- chromogenic culture medium) and treatment definition based on the results observed after 24 hours of incubation of the sample.

the responsible employee and the treatment was registered in the software used by the farm.

Treatment of clinical cases of mastitis

The treatment protocol for clinical cases of mastitis was defined by the veterinarian responsible for the farm. Four different pharmacological bases were used for the treatment with intramammary antibiotic therapy. The first option (ATB1) was the combination of oxytetracycline, neomycin, zinc bacitracin, and prednisone (Mastijet, MSD animal health, São Paulo, Brazil); the second (ATB2) was cephalexin-neomycin-prednisolone based (Masticine, MSD animal health, São Paulo, Brazil); the third was (ATB3) flumetasone-spiramycin-neomycin based (Flumast, Zoetis, São Paulo, Brazil) and the fourth option (ATB4) was cefoperazone-based (Mastclin, JA animal health, São Paulo, Brazil).

Treatment of clinical cases of mastitis was performed once a day, always after the end of the morning milking and according to the microbiological diagnosis (Figure 1). After milking the affected quarter, the tip of the teat was disinfected with 70% alcohol and the intramammary medication was infused, with upward massage for better distribution of the medication in the mammary gland.

All established treatments started with ATB1 during the first three days. If no clinical improvement was observed after three days, treatment with another pharmacological basis (ATB2) was started, and so on (ATB3 and ATB4). The CM were treated for two more consecutive days after no visible abnormalities in milk were observed. After this procedure, the cow entered the period of medication withdrawal until it was released. If the cow returned with clinical signs of mastitis during the waiting period, a new treatment was started with the next pharmacological basis of the protocol.

Data collection and variables

Milk production was measured every 15 d by an electronic scale (DeLaval Fi7, DeLaval, Missouri, USA). Individual milk samples were collected in vials containing bronopol (2-bromo 2-nitropropane 1,3-diol) for somatic cell count (SCC) analysis by flow cytometry (Somacount, Bentley Instruments Incorporated®, Iowa, USA).

Each mammary quarter affected was considered a clinical case of mastitis. If the clinical signs of mastitis disappeared and within 14 days the signs returned, this was then considered a recurrence (LAGO et al., 2011a).

To calculate the monthly/annual clinical mastitis incidence rate, we considered the total number of clinical cases in the month/year divided by the total number of lactating cows in the period (RUEGG, 2011).

Clinical cure was considered when there were no clinical symptoms within 14 days. It was considered that there was no cure in three different situations: when a case was repeated in the same quarter in less than 14 days, when it was necessary to dry the quarter with mastits, or when the cow was dried-off or discarded due to the clinical case.

The days in which the presence of changes in the milk of the evaluated case was observed were considered as days with clots in milk (DG).

The number of days of milk disposal was equal to the duration of clinical symptoms in the untreated group for mastitis. In the group treated for mastitis, DG was equal to the number of days in treatment plus four days after the last application of the drug, regardless of the antibiotic base used.

The incidence of clinical mastitis was considered as the number of clinical cases divided by the total number of teats at risk during lactation (305 days).

Financial analysis tool

Two simulations were proposed for financial evaluation (scenarios 1 and 2). Scenario 1 compared the situation if all cases of CM were treated (not using on-farm culture, i.e., treating all cows with clinical mastitis) *vs*. the use of on-farm culture (real data) and the savings generated for one year. Scenario 2 used real results with the savings generated by on-farm culture *vs*. the ideal treatment recommendation (Figure 1).

A total of 636 cows and 1,917 cases of mastitis were considered (values observed throughout the study).

The animals' lactation curve was calculated according to WOOD, (1967):

YT = axt (b) exp (-cxt), where:

YT = milk yield (kg);

a = parameter associated with the start of lactation;

b = represents the ascending phase of the curve;

c = represents the descending phase of the curve;

t = lactation time (days);

exp = constant with a value of 2.7182.

The lactation curve was used to measure milk loss after the occurrence of the clinical case of mastitis. For the calculation, the cows were separated into primiparous and multiparous, considering the peak yield, length, and persistency of lactation. The average number of days on which the first clinical case occurred during lactation was considered for future milk loss. The total production of primiparous and multiparous

(6,846 and 7,706 liters of milk, respectively) was used for the total production in 305 days.

The investment in fixed assets to adopt the technique was budgeted at $1,147.3, and included an incubator, a chair, a laboratory bench, and a Bunsen burner, among others. The total operating cost of treating clinical cases of mastitis was calculated by using the adapted methodology of MATSUNAGA et al. (1976). This cost was composed of variable operating costs and fixed operational costs, based on the results observed in the present study (Table 1).

The variable operating costs were composed of costs for feeding (of the treated cows) and direct costs for the treatment of clinical mastitis. These would be loss of milk during lactation, milk disposal, veterinarians, treatment of clinical mastitis, labor force, replacement of animals after culling or death and, costs with materials used in the laboratory (Table 2). The total amount of intramammary antibiotics used in each situation and the reduction in the use of antibiotics after the implementation of microbiological on-farm culture were also considered.

The average price paid per liter of milk in the state of Minas Gerais obtained by CEPEA (2018 and 2019 - R$ 1.50 = US$ 0.344) and the food cost for producing a liter of milk were taken into account for the calculation of milk disposal (Table 2). The cost of animal replacement was estimated by the cost of a heifer at calving (US$ 1,032.58), the sale of a cull cow (US$ 413.03), and the mortality rate, both values obtained in the local market and converted to the dollar of the day 18/02/20 (US$ 4,358).

The fixed operating costs were composed of the depreciation of the equipment used in the laboratory by the linear method.

Statistical analyses

Microsoft Excel® software was used to perform the analyzes. A descriptive analysis of the data related to the clinical case of mastitis was performed (grade, bacterial diagnosis, treatments performed, incidence of clinical mastitis, clinical cure rate, and days with clots in milk). Excel was used to develop the economic tool and to describe the results of the financial analysis.

RESULTS

Data from 1,287 cows were evaluated and the following data were removed from the analysis: data from animals with errors in data entry in the software on the farm (34 cows); animals with lactation greater than 720 days (5 cows); cows that did not show mastitis and milk weights in the first 60 days of lactation (21 cows). Thus, the total number of cows evaluated was 1,227 (1,582 lactations), divided into 28.0% first-calf cows (443 lactations), 27.8% second-calf cows (440 lactations), and 44.2% cows with more than three lactations (699 lactations).

The geometric mean of the bulk tank SCC from April to December (2018) was 349 x 10^3 cells/mL, and from January to September (2019) it reached 256 x 10^3 cells/mL (Figure 2).

Dynamics of infection

From the 1,582 lactations (1,227 animals), 1,917 cases of clinical mastitis were recorded (636 animals and 763 lactations), with a mean cumulative incidence of 48.2%. The monthly average incidence of clinical cases of mastitis was 4%, with june 2018 having the highest occurrence (7%) and August 2019

Table 1 - Parameters used and values for entry in the economic analysis.

Model input parameters	--------No on-farm culture------		-----------On-farm culture----------	
	Primiparous	Multiparous	Primiparous	Multiparous
Cases treated with antibiotics (%)	100	100	84	81
Cases tested in the farm culture (%)	-	-	91	91
Loss of production after the 1st case of mastitis (%)	5	5	5	5
Intramammary treatments performed (nº)	6.6	6.6	6.6	6.6
Disposal of milk from cows under treatment (average days)	9.8	9.8	9.2	9.2
Disposal of milk from untreated cows (average days)	-	-	6.2	6.2
Disposal of milk due (days)	4	4	4	4
Probability of removal from the herd to clinical mastitis (%)	3	9	3	9

Table 2 - Value used in each parameter for entry in the economic analysis.

Input parameters in the model	Value
Hourly value of the worker – Milker (US$)	12.5
Time spent treating mastitis (min/cow/day)	3.0
Average milk price - CEPEA MG 2018-2019 (US$/kg)	0.3
Feed cost (US$/liter/milk)	0.16
Veterinary cost (US$/work day)	229.5
Veterinary time spent during the day of service for mastitis treatment (%)	5.0
Average cost of intramammary antibiotic (US$/tube)	3.5
Support treatment cost when needed (US$/cow/case)	2.3
Average culture plate cost (US$/case)	1.0
Laboratory technician time (US$)	2.7
Cost for laboratory construction (Incubator, bench and other objects) (US$)	1,147.3
Depreciation time (years)	10.0
Residual value of the laboratory (US$)	114.7
Cost of laboratory supplies (US$/cow)	0.2
Time taken to perform the plate culture (for case/min)	3.0

having the lowest occurrence (2%) (Figure 3). The first case occurred on average at 125 days postpartum.

A total of 76.8% (1,473 cases) were classified as grade 1 mastitis; 20% (383 cases) were grade 2 mastitis, and only 3.2% (61 cases) were classified as grade 3 mastitis. From the 763 lactations with cases of clinical mastitis in the period evaluated, 40.5% (309 lactations) presented only one case of mastitis, 21.8% of lactations (166 lactations) had two cases of mastitis, and 37.7% (288 lactations) had three or more cases of mastitis.

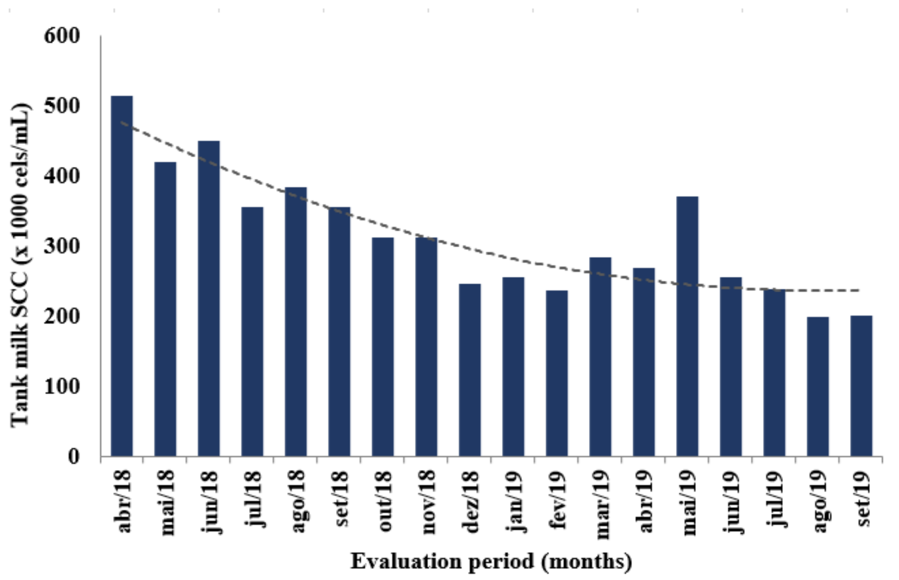

Figure 2 - Evaluation of somatic cell count (SCC x 1000 cels / mL) of milk in the tank during the evaluation period (18 months). Dotted line represents trend.

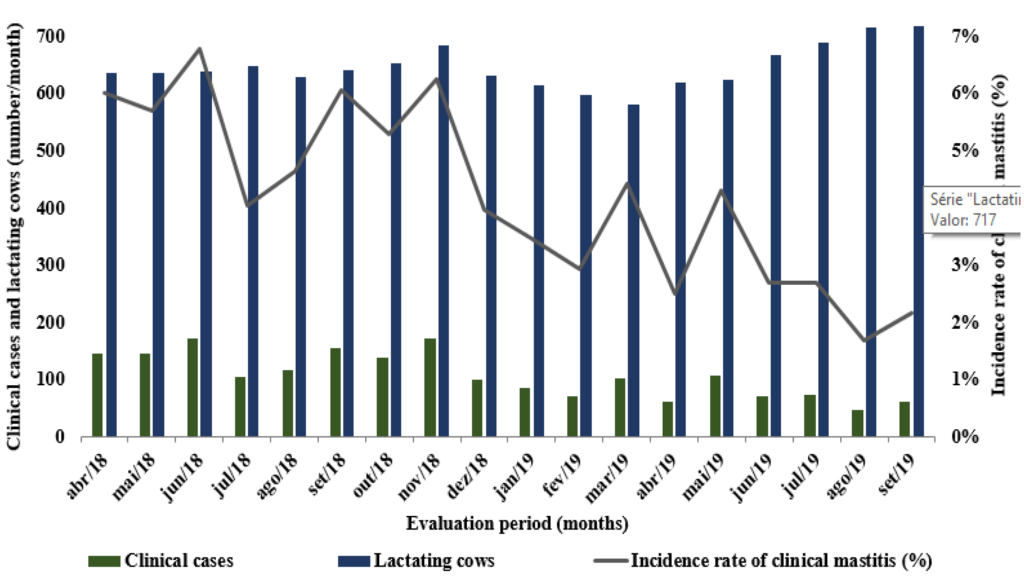

Figure 3 - Evaluation of the number of clinical cases, number of lactating cows and incidence rate of clinical mastitis during the evaluation period (18 months).

The incidence of clinical cases of mastitis in the entire period evaluated was 4.17% of new cases per month (107 new cases of mastitis per month in 645 lactating cows, Figure 3). In June 2018, a 7% incidence was recorded as the highest value observed during the evaluation period. The lowest value (2%) was observed in August and September 2019 (Figure 3).

Prevalence of microorganisms and treatment recommendation after plate reading

Throughout the evaluation period, 91.5% (1,754/1,917) of clinical mastitis cases had samples collected and inoculated in the plates. After 24 hours of incubation, the plates were evaluated, and the cases received treatment. Of the clinical cases, 8.5% (163/1,917) were not analyzed for unknown reasons.

In plate 1, 339 samples of clinical mastitis cases were inoculated. Microbial growth was reported in 77.3% (262/339) of the samples, while 21.8% (74/339) showed no growth, and 0.9% (3/339) showed contamination (Figure 4). In plate 2, samples from 1,142 cases of clinical mastitis were inoculated. Of the total, 51.5% (588/1142) showed bacterial growth but were not identified, while 24.3% of the samples (278/1,142) showed growth of *Streptococcus spp.*; 15% (171/1,142) did not show bacterial growth and 5.1% (58/1,142) showed Gram-negative bacterial growth (Figure 4). In plate 3, 273 samples were analyzed. A total of 53.1% (145/273) of the samples did not show bacterial growth. The most prevalent microorganisms reported in the samples that showed bacterial growth (46.9%, 128/273) were: *E. coli* (12.1%, 33/273), *non-aureus Staphylococci* (7.3%, 20/273), *Streptococcus* spp. (7.3%, 20/273), and *Streptococcus uberis* (6.2%, 17/273) (Figure 4).

From the total samples analyzed in the three plates, 27.8% (488/1,754) of the cases received a recommendation to not be treated and 72.2% (1,266/1,754) received a recommendation of treatment. In plate 1, 22.4% (76/339) and 77.6% (263/339) had the recommendation of no treatment and treatment, respectively. In plate 2, 20.3% (232/1,142) and 79.7% (910/1,142) had the recommendation of no treatment and treatment, respectively. In plate 3, 65.9% (180/273) and 34.1% (93/273) had the recommendation of no treatment and treatment, respectively.

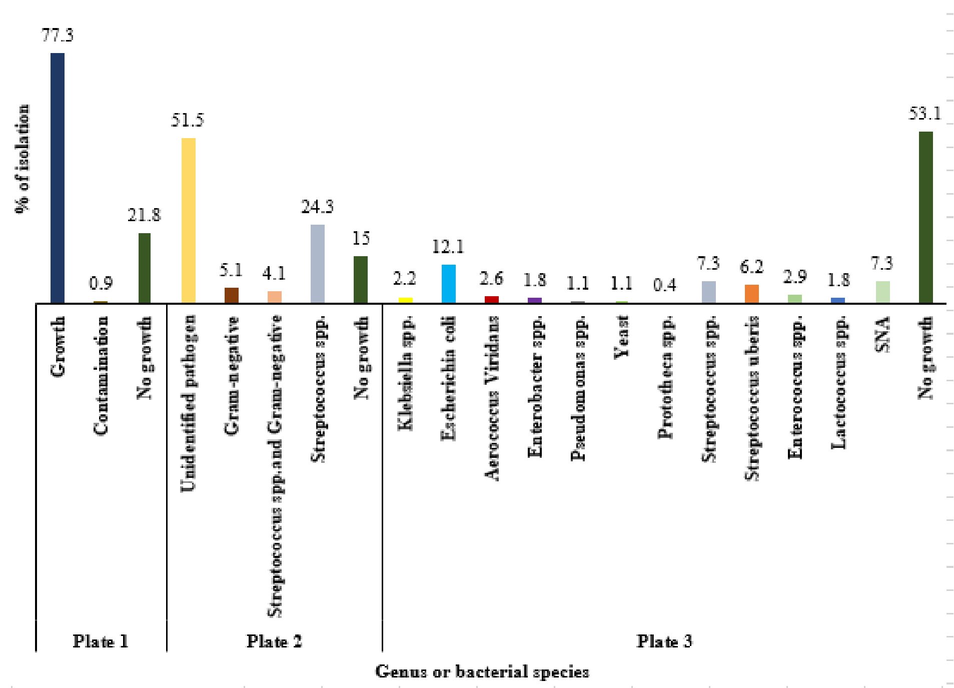

Figure 4 - Bacterial isolation (%) of clinical cases of mastitis in the different plates used, plate 1 (Agar), 2 (Tripartite) and 3 (Chromogenic).

Follow-up after treatment recommendation

Despite the laboratory's non-treatment recommendations for 27.8% of cases, only 18.6% (327/1,754) went untreated. As a result, the number of treated cases was 9.2% greater than expected, totaling 81.4% (1427/1,754). When performing this evaluation in each plate, it was reported that only 9.4% (32/339) of the cases in plate 1 were not effectively treated by using on-farm culture, while the number was 12.4% (142/1,142) in plate 2, and 56% (153/273) of the cases in plate 3.A total of 2.271 cases were treated with intramammary antibiotic therapy. ATB1 was used in 64.1% of the cases (1,455/2,271), while ATB2 was used in 19% (431/2,271), ATB3 in 11% (250/2,271), and ATB4 was used in 5.9% of the cases (135/2,271).

Clinical healing

Regarding the cases that followed the instruction of not being treated, 96.9% (31/32) of the cases in plate 1 were considered cured and only 3.1% (1/32) were not cured. For plate 2, 81% (115/142) were considered cured and 19% (27/142) were not cured, and as for plate 3, 84.3% (129/153) were considered cured and 15.7% (24/153) were not cured.

For the clinical cases that received treatment, 86% (264/307) in plate 1 were considered cured and 14% (43/307) were not cured. For plate 2, 83% (830/1,000) were considered cured and 17% (170/1,000) were not cured. At last, 86.7% (104/120) in plate 3 were considered cured and 13.3% (16/120) were not cured.

When assessing the clinical cure rate according to the intramammary antibiotic therapy performed, the ATB1, ATB2, ATB3, and ATB4 had clinical cure rates of 69.8% (1016/1455), 37.1% (160/431), 36.4% (91/250), and 59.3% (80/135), respectively.

Days with clots in milk, duration of the clinical case, and count of treatments performed

Clinical cases that did not receive intramammary antibiotic therapy (327/1754) presented a clinical cure rate of 84.3% (291/345) with an average of 3.2 days with clots in milk. Uncured cases totaled 15.7% (54/345) with an average of 9.6 days with clots in milk.

For cases that received intramammary antibiotic therapy, the clinical cure rate was 84%

(1320/1,572) with an average of 5 days with clots in milk. Meanwhile, 16% of the cases had no clinical cure (252/1,572) and had an average of 10.3 days with clots in milk.

On average, 6.6 intramammary treatments/CM were performed, with only 5.6 applications being used in the group that presented clinical cure, and 12 treatments in the group that did not present clinical cure.

Financial analysis

In scenario 1, after the implementation of on-farm culture (Table 3), the cost of the clinical case decreased by 10.3% (from US$99.82 to US$89.45). Costs related to treatment decreased by $5.451,12. This reduction represented savings of US$8.26 per lactating cow/year or US$4.28 per clinical case. Milk disposal costs decreased by $8.35 per cow/year or $4.23 per clinical case. Additionally, it was possible to observe an 18.4% of reduction in antibiotics used in the treatment of mastitis (Table 4).

In scenario 2, the real situation was compared to the ideal laboratory recommendation. For this scenario, only the number of cows that were left untreated was changed in the model (28.5%). Thus, in the ideal situation where all recommendations are adopted, it was possible to observe a 5.5% reduction in the total operating cost of the clinical case. This reduction represented savings of US$4.44 per cow/year or US$2.25 per clinical case, due to lower costs with intramammary antibiotic therapy and labor for treatment. The use of antibiotics to treat mastitis decreased by 11.8% in this scenario (Table 4).

DISCUSSION

The annual incidence of mastitis can vary from 13 to 40% and is related to the climatic and environmental conditions of the system used (MCDOUGALL et al., 2007; BAR et al., 2008; OLDE RIEKERINK et al., 2008). The annual incidence of clinical mastitis observed in our study (48%) is relatively high and it is possibly associated with the type of production system and management conditions of the farm. The greatest challenge in the semi-intensive system occurs during the wet season (high temperature and humidity), which increases the degree of exposure of the teats to organic matter in the animals' environment, leading to an increased risk of developing a clinical case of mastitis (HOGAN &

Table 3 - Costs associated with losses, treatment and laboratory in both evaluated scenarios, scenario 1 (No culture on-farm) and scenario 2 (Ideal current situation), compared to the current situation.

Costs	----Current situation----		---------Scenario 1[1]--------- -----No culture on-farm-----		-------------Scenario 2[2]---------- -----Ideal current situation-----	
	US$/Year	%	US$/Year	%	US$/Year	%
----Associated losses----						
Loss of production	12,655.1	11.1	12,655.11	9.9	12,655.1	11.7
Milk Disposal	60,091.0	52.7	69,617.7	54.7	56,703.1	52.6
Death replacement and disposal costs	15.0	13.2	15.0	11.8	15.0	13.9
Veterinary costs	298.9	0.3	298.9	0.2	298.9	0.3
----Treatment----						
Medication and support costs	24,009.2	21.1	29,404.5	23.1	21,170.9	19.6
Cost of labor for treatment	248.5	0.2	304.5	0.2	219.1	0.2
----Laboratory----						
Costs with laboratories and supplies	1,697.7	1.5	0.0	0.0	1,697.7	1.6
Total cost of mastitis per year	114,0	100	127.3	100	107.7	100

[1]Scenario 1: compared the situation if all cases of CM were treated (not using on-farm culture, i.e., treating all cows with clinical mastitis) *vs*. the use of on-farm culture (real data) and the savings generated for one year;

[2]Scenario 2: used real results with the savings generated by on-farm culture *vs*. the ideal treatment recommendation.

Table 4 - Costs associated with the clinical case of cow mastitis / case and reduction in the use of antibiotics, in both evaluated scenarios, scenario 1 (no culture on-farm) and scenario 2 (ideal current situation) compared to the current situation.

Items	Current situation	-------Scenario 1------- No culture on-farm	-------Scenario 2------- Ideal current situation
Total cost of mastitis (US $ / cow / year)	172.0	197.0	166.8
Cost per case of mastitis (US $ / case)	89.5	99.9	84.6
Cost of using antibiotics (US $)	1,575.5	1,929.3	1,389.2
Total difference in cost of mastitis per cow / year (US $)	-	20.5	9.7
Reduced use of antibiotics (%)	-	18.4	11.8

[1]Scenario 1: compared the situation if all cases of CM were treated (not using on-farm culture, i.e., treating all cows with clinical mastitis) *vs*. the use of on-farm culture (real data) and the savings generated for one year.
[2]Scenario 2: used real results with the savings generated by on-farm culture *vs*. the ideal treatment recommendation.

SMITH, 2012; OLIVEIRA et al., 2015). Moreover, animals are more susceptible to heat stress, which directly affects the immune system and makes animals susceptible to intramammary infection (HOGAN & SMITH, 2012).

Most clinical cases of mastitis were classified as grade 1 (76.8%) and only 20% as grade 2. PINZON-SANCHEZ & RUEGG (2011) and OLIVEIRA et al. (2013) observed an occurrence of 27 - 35% of grade 2 mastitis in free-stall confined cows. The reduction observed in our study may be related to the vaccination strategy used by the farm. Vaccination against *E. coli* induces the production of antibodies against a wide variety of gram-negative bacteria (CHAIYOTWITTAYAKUN et al., 2004). Even though vaccination does not protect against infection, it has been identified as capable of reducing the clinical symptoms of clinical cases, which may justify the results observed in our study (GONZÁLEZ et al., 1989; MAIA et al., 2013).

There was a decrease in the rate of new infections throughout the evaluation period, implying a lower incidence rate of clinical mastitis in the last months of the study. The observed values are within the values established by RUEGG, 2011 (3 - 4%). There was also a reduction in the SCC of the tank milk to values < 250,000 cells/mL. These results showed that the farm has improved control during this period, with improvement in the environment in which cows are kept, labor training, and establishment of culture and targeted treatment of clinical cases of mastitis.

A total of 27.8% of the clinical cases evaluated received the recommendation of no treatment, which is below the results reported by other authors that ranged from 46 to 68.5% (OLDE RIEKERINK et al., 2008; LAGO et al., 2011a; VASQUEZ et al., 2017). This low number of non-treatments occurred mainly when using the blood agar plate (22.4%) and the tri-plate (20.3%). Interestingly, it was decided to use the tri-plate in order to increase the number of clinical cases that should not be treated due to the possibility of isolation of Gram-negative bacteria; however, this increase was not observed in our study. Both plates have non-selective culture media, and the growth of undesirable bacteria is common in cases of errors during inoculation. Growth of these bacteria may have occurred during the study, which resulted in the largest number of treated clinical cases.

In contrast, there was an increase in the number of clinical cases with indication of non-treatment (65.9%) with the use of the Chromogenic plate, possibly due to the greater selectivity of the medium used. The plate used has an accuracy of 90.5%, sensitivity of 97.6%; specificity of 85.8%, negative predictive value of 98.2%, and positive predictive value of 82% (FERREIRA et al., 2018). Similar results were reported by ROBERSON, (2003) and LAGO et al. (2011 a) with nearly 50 - 80% and 56% of cases with a recommendation not to be treated, respectively.

The use of the Chromogenic plate enabled the identification of 36.6% of environmental pathogens (coliforms and *Streptococcus*), representing 70.9% of the total bacteria that grew on the plates. Over the years, the farm has intensified the control of contagious mastitis through basic control measures in addition to the elimination of cows carrying *Streptococcus agalactiae* and *Staphylococcus aureus*. There is a high prevalence of intramammary infections by environmental microorganisms in herds in which contagious pathogens are effectively controlled (HOGAN et al., 1988).

In addition, 53.1% of the samples did not show bacterial growth when using the chromogenic plate. This percentage exceeds the values observed by ROBERSON, (2003) and LAGO et al. (2011 a). In the present study, plate reading was performed 24 hours after incubation. According to LAGO & GODDEN, (2018), the plates with no bacterial growth should remain incubated for another 24 hours after reading due to the possibility of *Corynebacterium bovis* growth in this period. However, this procedure was not common to be performed on farms. The increase in incubation time and the consequent delay in starting treatment can compromise the cure rate in addition to animal welfare. Furthermore, *Mycoplasma* spp. is an important causative agent of mastitis in herds and its prevalence has been under estimated as it requires a specific culture medium for isolation (PRETTO et al., 2001).

In our study, only 18.6% of cases were left untreated despite the recommendations for no treatment for 27.8% of cases obtained after reading the plates. In the short term, it is common for employees to fear not carrying out the treatment of a clinical case after the implementation of the on-farm culture system, which usually results in a greater number of treatments. With the intensification of training and the employees' awareness of following the laboratory's guidelines, it was possible to observe evolution in these results.

The clinical cure rate was numerically similar for treated (84.3%) and untreated (84%) clinical cases. This is a case study, not designed with the objective to compare the efficiency of the different treatments used in the farm. However, regardless of the pharmacological base used or the number of treatments performed, we observed a clinical cure rate higher to 84%. According to WENZ et al. (2001) and LANGONINI, (2013), it is important to have as a goal a clinical cure rate greater than 80% in order to assess the efficiency of the treatments performed.

Regarding the number of days with DG, the untreated cases show an average of 4.2 days (3.2 and 9.6 days for clinically cured and uncured cases, respectively). Results similar to those were observed by HOE & RUEGG, (2005) and FUENZALIDA & RUEGG, (2019), of 4.1 and 4.2 days, respectively, and slightly higher than that found by LAGO et al. (2011 a), of 3 days. For the treated clinical cases, the average number of days with clots in milk was 5.9 days, which is higher than that observed by LAGO et al. (2011 a) and VASQUEZ et al. (2017), of 2.7 and 4.5 days, respectively.

There was no control of experimental groups in our study, therefore, it was not possible to select cows with concomitant diseases that could be excluded from the work, as performed by LAGO et al. (2011 a). In addition, another factor that must be considered is that mastitis treatment decisions on farms are based on protocols without technical considerations, which can somehow lead to reduced treatment efficiency, increased costs, and the risk of antibiotic residues in the milk (OLIVEIRA & RUEGG, 2014).

Lastly, our financial analysis has demonstrated that it is economically feasible to implement on-farm culture to guide clinical mastitis treatment decision. The cost per clinical case was US$99.9 in scenario 1. Similar values were found by OLIVEIRA et al. (2012), who reported an average cost of US$70.78 per clinical case of mastitis (data deflated by the IGP-DI to actual values for this study's date). GUIMARÃES et al. (2017) reported an economic impact of US$107.10 per clinical case (deflated values) but used the cost of subclinical mastitis in the calculation. The total cost of a clinical case is variable and highly dependent on the methodology used to perform the calculations (DOWN et al., 2013).

Milk disposal represented 54.7% of the total operating cost of clinical cases. OLIVEIRA et al. (2012) and HALASA et al. (2007) also reported the disposal of milk as the most significant cost. The disposal of milk is of greater economic importance than the loss of production during lactation, as there are associated costs for its production (PETROVSKI et al., 2006; HALASA et al., 2007). The treatment cost was the second item that most impact the total operating cost of clinical cases (23.3%).

When considering these two main costs in scenario 1 and evaluating the financial results after the implementation of on-farm culture, a reduction of only 3.6% in the costs of milk disposal and 8.6% in relation to the treatment was observed. When considering all costs associated with treatment, there was a 10.4% reduction after the culture was implemented. This small reduction is due to clinical cases that had no recommendation to be treated (27.8%), and only 18.6% went untreated. As a result, the number of treatments was 9.2% greater than expected if treatment recommendations were followed, with a consequent increase in costs with milk disposal and treatment.

If the treatment recommendations were followed correctly by the farm (scenario 2), it would be possible to observe a reduction of 5.5% in the total operating cost of the clinical case. However, the use of the on-farm technique would be justified by the reduction in the use of antibiotics.

The results of this study help assessing costs related to the treatment of clinical mastitis. They can still change depending on the milk price, feed cost, days in milk when the clinical case of mastitis occurred, culling or death of the cow, and treatment costs (BAR et al., 2008). In order to implement the on-farm culture of clinical cases, it is important to assess and identify the pathogens associated with clinical cases of mastitis. The predominance of Gram-positive bacteria, results in a greater number of intramammary antibiotic treatments, and depending on the prevalence of these pathogens in the herd, the availability of this tool may be questionable (DOWN et al., 2017).

CONCLUSION

The implementation of on-farm culture enhanced treatment accuracy of clinical cases of mastitis, with a reduction of up to 28% in the use of antibiotics. In the medium term, the use of the technique resulted in a reduction in the SCC of the tank milk and the non-treatment of some cases did not affect the rate of clinical cure. The costs of clinical cases of mastitis can be reduced by 10.4% after on-farm culture has been implemented. However, the microbiological profile of clinical cases of mastitis must be considered for the use correct definition of treatment protocols.

ACKNOWLEDGMENTS

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, Brasil), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Minas Gerais, Brasil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brasil), and Escola de Veterinária da Universidade Federal de Minas Gerais (UFMG, Minas Gerais, Brasil). The authors would like to thank the Farm and IDEAGRI® for making the data available for the study.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

BAR, D. et al. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. **Journal of Dairy Science**, v.91, p.2205-2214, 2008. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(08)71171-8/abstract>. Accessed: Jan. 4, 2021. doi: <https://doi:10.3168/jds.2007-0573>.

CHAIYOTWITTAYAKUN, A. et al. Hyperimmunization of steers with J5 *Escherichia coli* bacterin: effects on isotype-specific serum antibody responses and cross reactivity with heterogeneous gram-negative bacteria. **Journal of Dairy Science**, v.87, p.3375-3385, 2004. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(04)73473-6/fulltext>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.S0022-0302(04)73473-6>.

CHAKRABORTY, S. et al. Technological interventions and advances in the diagnosis of intramammary infections in animals with emphasis on bovine population- a review. **Veterinary Quarterly**, v.39, v.76-94, 2019. Available from: <https://www.tandfonline.com/doi/full/10.1080/01652176.2019.1642546>. Accessed: Jan. 4, 2021. doi: <https: // doi: 10.1080 / 01652176.2019.1642546>.

DOWN, P.M. et al. Factors affecting the cost-effectiveness of on-farm culture prior to the treatment of clinical mastitis in dairy cows. **Preventive Veterinary Medicine**, v.145, p.91-99, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S0167587717302684>. Accessed: Jan. 4, 2021. doi: <https: // doi: 10.1016 / j.prevetmed.2017.07.006>.

DOWN, P.M. et al. Rate of transmission: a major determinant of the cost of clinical mastitis. **Journal of Dairy Science**, v.96, p.6301-6314, 2013. Available from: <https://doi.org/10.3168/jds.2012-6470>. Accessed: Jan. 4, 2021. doi: <https://www.sciencedirect.com/science/article/pii/S0022030213005559>.

FERREIRA, J. C. et al. Comparative analysis of four commercial on-farm culture methods to identify bacteria associated with clinical mastitis in dairy cattle. **PloS one**, e0194211, 2018. Available from: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0194211>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1371/journal.pone.019421>.

FISCHER, K. et al. Dairy farmers' perspectives on antibiotic use: a qualitative study. **Journal of Dairy Science**, v.102, p.2724-2737, 2019. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(19)30006-2/abstract>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2018-15015>.

FUENZALIDA, M. J.; RUEGG, P. L. Negatively controlled, randomized clinical trial to evaluate intramammary treatment of nonsevere, gram-negative clinical mastitis. **Journal of Dairy Science**, v.102, p.1-20, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030219303492>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2018-16156>.

GONZÁLEZ, R. N. et al. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. **Canadian Journal of Veterinary Research**. v.53, p.301-305, 1989. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1255715/>. Accessed: Jan. 4, 2021.

GUIMARÃES, J. L. B. Estimate of the economic impact of mastitis: A case study in a Holstein dairy herd under tropical conditions. **Preventive Veterinary Medicine**, v.142, p.46-50, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0167587716307115>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1016/j.prevetmed.2017.04.011>.

HALASA, T. Economic effects of bovine mastitis and mastitis management: A review. **Veterinary Quarterly**, v.29, p.8-31, 2007. Available from: <https://www.tandfonline.com/doi/abs/10.1080/01652176.2007.9695224>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1080/01652176.2007.9695224>.

HOE, F. G.; RUEGG, P. L. Relationship between antimicrobial susceptibility of clinical mastitis pathogens and treatment outcome in cows. **American Veterinary Medical Association**. v.227, p.1461-1468, 2005. Available from: <https://avmajournals.avma.org/doi/abs/10.2460/javma.2005.227.1461>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.2460/javma.2005.227.1461>.

HOGAN, J.; SMITH, K. L. Managing environmental mastitis. **Veterinary Clinics of North America: Food Animal Practice**, v.28, p.217-224, 2012. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0749072012000266?via%3Dihub>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1016/j.cvfa.2012.03.009>.

HOGAN, J. S. Growth response of environmental mastitis pathogens to long-chain fatty acids. **Journal of Dairy Science**. v.71, p.245-249, 1988. Available from: <https://www.sciencedirect.com/science/article/pii/S002203028879548X>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.S0022-0302(88)79548-X>.

LAGO, A.; GODDEN, S. M. Use of rapid culture systems to guide clinical mastitis treatment decisions. **Veterinary Clinics of North America: Food Animal Practice**, v.34, p.389-412, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0749072018300306?via%3Dihub>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1016/j.cvfa.2018.06.001>.

LAGO, A. et al. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. **Journal of Dairy Science**, v.94, p.4441-4456, 2011a. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(11)00456-5/fulltext>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2010-4046>.

LAGO, A. et al. The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. **Journal of Dairy Science**, v.94, p.4457-4467, 2011b. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(11)00457-7/fulltext>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2010-4047>.

LANGONINI, H. Milk quality: utopia without a serious program to monitor the occurrence of bovine mastitis. **Pesquisa Veterinária Brasileira**, v.33, p.620-626, 2013. Available from: <https://www.scielo.br/j/pvb/a/Z5TcnvtpCJwTqLrnyHmjwQN/?lang=pt>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1590/S0100-736X2013000500012>.

MAIA, P. V. et al. Pre-delivery *Escherichia coli* J5 vaccination and occurrence of mastitis and milk production of crossbred dairy cows. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.65, p.1367-1375, 2013. Available from: <https://www.scielo.br/j/abmvz/a/4DxZg8kwv7nqw9sKhW9pkQj/?lang=pt>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1590/S0102-09352013000500014>.

MANSION-DE VRIES, E. M. et al. A field study evaluation of Petrifilm™ plates as a 24-h rapid diagnostic test for clinical mastitis on a dairy farm. **Preventive Veterinary Medicine**, v.113, p.620-624, 2014. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S016758771300370X?via%3Dihub>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1016/j.prevetmed.2013.11.019>.

MATSUNAGA, M. et al. Production cost methodology used by the IEA. Agriculture in São Paulo. v.23, p.123-139, 1976.

MCDOUGALL, S. Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. **New Zealand Veterinary Journal**, v.55, p.161-170, 2007. Available from: <https://www.tandfonline.com/doi/abs/10.1080/00480169.2007.36762>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1080/00480169.2007.36762>.

OLDE RIEKERINK, R. G. M. et al. Incidence rate of clinical mastitis on canadian dairy farms. **Journal of Dairy Science**, v.91, p.1366-1377, 2008. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030208712645>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2007-0757>.

OLIVEIRA, C. S. F. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. **Preventive Veterinary Medicine**, v.121, p.297-305, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S0167587715002652>. Accessed: Jan. 4, 2021. doi: <https://doi: 10.1016 / j.prevetmed.2015.08.001>.

OLIVEIRA, C. S. F. **Epidemiological and bioeconomic analysis of bovine mastitis in Brazilian herds**. 2012. 86f. Thesis (Doctorate in Veterinary Medicine) - Veterinary School, Federal University of Minas Gerais, Belo Horizonte.

OLIVEIRA, L.; RUEGG, P. L. Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. **Journal of Dairy Science**, v.97, p.5426-5436, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030214004639>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2013-7756>.

OLIVEIRA, L. et al. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. **Journal of Dairy Science**, v.96, p.7538-7549, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S002203021300670X>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2012-6078>.

PETROVSKI, K. R. et al. A review of the factors affecting the costs of bovine mastitis. **Journal of the South African Veterinary Association**, v.77, p.52-60, 2006. Available from: <https://jsava.co.za/index.php/jsava/article/view/344>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.4102/jsava.v77i2.344>.

PINZÓN-SÁNCHEZ, C. et al. 2011. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. **Journal of Dairy Science**, v.94, p.1873-1892, 2011. Available from: <https://pubmed.ncbi.nlm.nih.gov/21426977/>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2010-3930>.

POL, M.; RUEGG, P. L. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. **Journal of Dairy Science**, v.90, p.262-273, 2007.

Available from: <https://www.sciencedirect.com/science/article/pii/S0022030207726279>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.S0022-0302(07)72627-9>.

PRETTO, L. G. et al. Bovine mastitis in dairy cows caused by *Mycoplasma bovis*. **Pesquisa Veterinária Brasileira**, v.21, p.143-145, 2001. Available from: <https://www.scielo.br/j/pvb/a/snNVBPtBVYQ46KGYtCk6MLv/?lang=pt>. Accessed: Jan. 4, 2021. doi: <http://dx.doi.org/10.1590/S0100-736X2001000400003>.

PYÖRÄLÄ, S. et al. Efficacy of two therapy regimens for treatment of experimentally induced *Escherichia coli* mastitis in cows. **Journal of Dairy Science**, v.77, p.453-461, 1994. Available from: <https://www.sciencedirect.com/science/article/pii/S0022030294769733>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.S0022-0302(94)76973-3>.

ROBERSON, J. R. Establishing treatment protocols for clinical mastitis. 2003. **Veterinary Clinics of North America: Food Animal Practice**, v.19, p.223-234, 2003. Available from: <https://www.vetfood.theclinics.com/article/S0749-0720(02)00071-3/abstract>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.1016/s0749-0720(02)00071-3>.

RUEGG, P. L. Managing mastitis and producing high quality milk. Wiley-Blackwell Publishing, LTD, Hoboken, NJ. 2011. Cap.18, p.207-232. doi: <https://doi.org/10.1002/9780470960554.ch18>.

VASQUEZ, A. K. et al. 2017. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. **Journal of Dairy Science**, v.100, p.2992-3003, 2017. Available from: <https://www.journalofdairyscience.org/article/S0022-0302(17)30090-5/abstract>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.3168/jds.2016-11614>.

WENZ, J. R. Use of systemic disease signs to assess disease severity in dairy cows with acute coliform mastitis. **Journal of the American Veterinary Medical Association**, v.218, p.567-572, 2001. Available from: <https://pubmed.ncbi.nlm.nih.gov/11229511/>. Accessed: Jan. 4, 2021. doi: <https://doi.org/10.2460/javma.2001,218,567>.

WOOD, P.D.P. Algebraic model of the lactation curve in cattle. **Nature**, v.206, p.164-165, 1967. Available from: <https://www.nature.com/articles/216164a0>. Accessed: Jan. 4, 2021.