Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

Animal Production and Environment
Received on: 28/04/2023
Accepted on: 21/07/2023

Cost-effectiveness of selective therapy for clinical mastitis based on on-farm pathogen identification

Custo-benefício da terapia seletiva para mastite clínica com base na identificação de patógenos na fazenda

[1] Santos, Ederson dos
https://orcid.org/0000-0003-1285-5412

[1] Xavier, Eduardo Gularte
https://orcid.org/0000-0001-7129-9212

[1] Komninou, Eliza Rossi
https://orcid.org/0000-0001-6591-408X

[1] Lima, Alexandre Martins
https://orcid.org/0000-0002-7197-9100

[1*] Teixeira, Ritieli dos Santos
https://orcid.org/0000-0002-0375-5308

[1] Corrêa, Marcio Nunes
https://orcid.org/0000-0003-0855-2750

[1] Barbosa, Antônio Amaral
https://orcid.org/0000-0001-6062-3486

[1] Schmitt, Eduardo
https://orcid.org/0000-0003-1900-3968

[1]Universidade Federal de Pelotas - UFPEL, Faculdade de Veterinária, Capão do Leão, 96160-000, RS/Brazil
*Mail for correspondence: rititeixeira@hotmail.com

ABSTRACT

This research aims to describe the cost-benefit analysis of selective therapy adoption for clinical mastitis based on the identification of pathogens using on-farm culture system. A survey of clinical mastitis cases was carried out in the database of a dairy herd with 500 lactating Holstein cows, during the period of September 2017 to October 2019. Variables such as frequency of cases, main pathogens identified, costs with antibiotic therapies and milk disposal were evaluated and compared between one year before (P1) and after (P2), the farm's selective therapy adoption. During the study period, 599 cases of clinical mastitis were identified, 361 in P1 and 238 in P2. The average cost of therapies was US74.33 and US91.82 for P1 and P2. Considering the two years of the study, the estimated cost of treating clinical mastitis was US45,444.31, of which US$27,559.97 were spent in P1 and US17,884.34 in P2. Based on the result of the identification of the pathogen types associated with clinical mastitis, selective therapy demonstrated cost reduction already in the first year of implementation.
Keywords: **A**ntimicrobial resistance, Diagnosis on-farm, Economic impact

RESUMO

Esta pesquisa objetivou descrever a análise custo-benefício da adoção de terapia seletiva para mastite clínica a partir da identificação de patógenos por meio da cultura em placas na fazenda. Foi realizado um levantamento de casos clínicos de mastite no banco de dados de uma fazenda com 500 vacas Holandesas em lactação, durante o período de setembro

RBSPA
Revista Brasileira de Saúde e Produção Animal
Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

de 2017 à outubro de 2019. Foram avaliadas as variáveis frequência de casos, principais patógenos identificados, custos com antibioticoterapia e descarte de leite foram, as quais foram comparados entre um ano antes (P1) e após (P2), à adoção da terapia seletiva na fazenda. Durante o período do estudo, foram identificados 599 casos de mastite clínica, sendo 361 em P1 e 238 em P2. O custo médio das terapias foi de US74,33 e US91,82 para P1 e P2. Considerando os dois anos de estudo, o custo estimado do tratamento da mastite clínica foi de US45.444,31, dos quais US27.559,97 foram gastos em P1 e US17.884,34 em P2. Com base na identificação dos tipos de patógenos associados à mastite clínica, a terapia seletiva demonstrou redução de custos já no primeiro ano de implantação.
Palavras chaves: Diagnóstico na fazenda, Impacto econômico, Resistência antimicrobiana

INTRODUCTION

Mastitis is considered the most critical disease in dairy herds and the one that provides the greatest economic losses in milk production systems worldwide (Ruegg, 2017). In addition to implications for animal welfare, the disease significantly reduces milk production. This factor plus to the costs of treatment of clinical mastitis, culling of infected animals, and milk disposal due to antibiotic residues are the main losses related to mastitis that impact dairy farms' profitability (Guimarães et al., 2017).
One of the measures of mastitis control is treating clinical mastitis cases with antibiotics. But, its use in therapies performed during lactation generates residues in the milk, and in some cases, it has a low cost-benefit ratio, which must be considered for treatment decisions (Pyörälä, 2009). Also, the use of antibiotics in the dairy farms can contribute to the emergence of multi-resistant bacteria (Manyi-Loh et al., 2018; Garcia et al. 2019; Gonçalves et al. 2020; He et al. 2020).
To reduce the use of antibiotics without reduction of cure rates of clinical mastitis in herds, a concept of targeted or selective therapy may be advantageous in comparison to the idea of conventional treatment (Schmenger et al., 2020). Commonly, when clinical mastitis is diagnosed but microbial culture is not performed, prompt treatment of all cases with broad-spectrum antibiotics is recommended (Vries et al., 2016), although only 10% to 40% cases justify such use (Roberson, 2003; Lago et al., 2011).
Despite Rio Grande do Sul is the third-largest milk-producing state by volume in Brazil, studies reporting cost-benefits of adopting selective therapy to treat clinical mastitis have not been found. The present study aimed to compare retrospective data from the year before adopting selective therapy and the year after its implantation in a large dairy herd in the southern Rio Grande do Sul, seeking to analyze its influence on economic and epidemiological components related to clinical mastitis treatment on farm.

MATERIALS AND METHODS

This study was carried out following the recommendations of the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (nº CEEA 14122-2020), on a dairy farm from Rio Grande do Sul state in Brazil. It has approximately 500 lactating Holstein cows and an average production of 14,040 liters/day (2019 data). Cows were housed in a Compost-bedded pack barn

Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

(CBP) system with mechanical milking, performed twice a day.

Information contained in the farm's database and weekly recorded by the veterinarian responsible for the health of dairy cattle was obtained by the software management Alpro DeLaval® (DeLaval, Tumba, Botkyrka, Sweden) and Ideagri® (Ideagri, Belo Horizonte, Minas Gerais, Brazil). Approximately data from 1500 animals from September 2017 to October 2019 were recovered from filtering the information of interest. These included: day, month, year, causative microorganism, type and duration of the treatments, and number of days of milk disposal. The differences found between the periods for the variables: frequency of use of antibiotics; costs with medicines and veterinary services; the volume of milk discarded; frequency of cases; primary pathogens involved; occurrence of relapses, and clinical recurrence, were computed for later use in the economic and epidemiological analyzes.

Aiming to collect data regarding therapies used between September 2017 to October 2019 to treat the cases of clinical mastitis, the total period was divided into two: period 1 (P1), from September 2017 to September 2018, in which the farm did not yet use microbiological culture on-farm and; period 2 (P2), from October 2018 to October 2019, when the microbiological culture was implemented to identify the pathogens associated to clinical mastitis and to adopt selective therapy as a strategy to clinical mastitis treatment decisions. Also, according to particularities, the two periods (P1 and P2) were further subdivided. In P1, based on traditional microbial culture and antibiogram results, the farm used broad-spectrum intramammary antibiotics (IMM). Those analyses were performed at the beginning of each year, sampling the entire herd through a sample collection from the bulk tank raw milk. Thus, considering that the therapeutic protocol used in 2017 was different from that used in 2018, these periods were designated as P1a and P1b, respectively. During P1a, treatments were performed by intramammary (IMM) administration of an association of tetracycline, neomycin, bacitracin, and prednisone (Mastijet®Fort - MSD Animal Health, Kenilworth, New Jersey, United States of America (USA) for four days (T1). In P1b, the IMM administration of ceftiofur (Spectramast®LC - Zoetis, Parsippany-Troy Hills, New Jersey, USA) was used for four days (T2).

Based on the result of on-farm cultures, at P2, the treatments were established according to the type of agent identified and the protocol established by the farm. From October 2018 to June 2019, the Accumast® (FERA Diagnostics and Biologicals, College Station, Texas, USA) plates were used, while from July to October 2019, the On farm® plates (Ag Tech, Piracicaba, São Paulo, Brazil). For this reason, we chose to designate such subperiods as P2a and P2b, respectively. In addition, three different types of treatments were established according to the pathogen identified in the microbiological analysis (T3, T4, and T5).

In P2 (P2a and P2b), mastitis caused by Gram-negative bacteria were treated for three days with the association of IMM ciprofloxacin (Ciprolac® - Ourofino Animal Health, São Paulo Brazil) and intramuscularly (IM) Enrofloxacin 10% (Fabiani Saúde Animal, São Paulo, Brazil) at 7.5 milligrams per kilo (mg / Kg) in 24-hour intervals (T3). Mastitis caused by Gram-positive bacteria were treated with a daily IMM administration of Ciprolac® for five days and three daily IM administration of Enrofloxacin 10%

ISSN 1519 9940

at 7.5 mg / Kg (T4). Mastitis caused by *Streptococcus agalactiae* and milder conditions were treated with daily IMM administration of Spectramast LC® for four days, and two doses of IM amoxicillin (Clamoxil®- Zoetis, Parsippany-Troy Hills, New Jersey, USA) at a dose of 15 mg / Kg, in intervals of 48 hours among administration (T5). Animals whose samples were positive for *Escherichia coli* or that did not present microbial growth in culture were not treated with antibiotics (Lago et al. 2011; Pinzón-Sánchez et al. 2011; Suojala et al. 2013). Exceptions such as mastitis classified as severe cases (grade 3: cows with systemic clinical signs including fever and poor appetite) were treated with T5 protocol plus supportive treatment (Schmenger et al. 2020).

In cases where it was not possible to perform the culture in plates due to unforeseen events such as the absence of the responsible professional, the therapeutic protocol for Gram-positive was adopted (73 cases, 12 of which were relapses and 11 were recurrences).

The on-farm microbiological culture systems *Accumast*® and *On farm*® had an average price of US5.09 per exam (considering the US1.00 = R3.53 (exchange quote used to calculate costs)). Samples were considered positive if one or more colonies grew after culture in the plate, while those that did not show growth were classified as negative (no growth). In addition to these data, animals that presented relapses and mastitis recurrence were also identified. The cases were considered relapse when there was a new case of clinical mastitis of the same cow within 21 days and recurrences when there was a new case 21 days later (Kumar et al., 2016).

The use of antibiotics was estimated based on the number of treated cases (Schmenger et al., 2020). To calculate the expenses with antibiotics (intramammary and injectable) used to treat clinical mastitis, an average price of three catalogs from different commercial companies was used for each subperiod of the study. The prices and doses of antibiotics used to quantify the cost of treating mastitis are presented in the Table 1.

Table 1 Values of doses used to quantify the cost of treating mastitis.

ANTIBIOTICS [1]	VALUE[2]
CLAMOXIL	$ 18.63
ENROFLOXACIN 10%	$ 8.15
MASTIJET FORT®	$ 16.72
CIPROLAC®	$ 19.69
SPECTRAMAST LC®	$ 11.09

[1]average dose used per animal
[2]average price of medications during the study period
P1a - September to December 2017; P1b - January to October 2018; P2a -October 2018 to June 2019, P2b - July to October 2019.

The cost of veterinary services for each case of mastitis was estimated at around US1.55, considering that for each clinical case of clinical mastitis, the professional took around 15 minutes to make the diagnosis and collect a sample to perform the microbiological culture. Also, the veterinarians hired through a Trainee Program on the farm had the monthly salary calculated at approximately US991.50 per month for an 8-hourworkload per day.

RBSPA Revista Brasileira de Saúde e Produção Animal
Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

To reach the cost of each treatment, the frequency of clinical mastitis cases during lactation was multiplied by the cost associated with the specific treatment (T1, T2, T3, T4, or T5). Also, the expense with veterinary services and the total of non-marketable or discarded milk due to antibiotic residues were assessed. The average cost for each study period was calculated considering the different periods, in P1 adding the values of each protocol (T1 and T2) and divided by 2, while in P2 dividing by the number of protocols.
The amount of milk discarded was analyzed based on the average dairy herd production for each study period (Table 2). According to the protocols adopted by the farm, in the treatment of clinical mastitis, seven days were necessary for the T3 protocols and eight days of milk disposal for the other protocols (6 and 7 days of disposal according to recommendations for the antimicrobials used, and another day of safety management, adopted by the farm to ensure that there was no residue in the milk. The milk produced during episodes of clinical mastitis and that after the end of treatment with antibiotics was considered non-marketable was subtracted from the total production of marketable milk from the herd (Shim et al., 2004). The price of milk used to perform the calculations was based on the average price established by the Rio Grande do Sul State Milk Producers / Industries Joint Council (CONSELEITE-RS) for the entire period study (Table 2). These values were approximately the average that the farm received per liter of milk sold in September to December 2017, throughout 2018, and from January to October 2019.

Table 2 Average production of liters per month and average milk price in each period.

YEAR	AVERAGE MILK / COW PRODUCTION / DAY	AVERAGE NUMBER OF LACTATING COWS	AVERAGE DAILY PRODUCTION OF THE FLOCK	LITER PRICE
2017	25.6 L	563	14,412.8 L	$ 0.27
2018	28.9 L	494	14,276.6 L	$ 0.27
2019	30 L	468	14,040 L	$ 0.30

Source milk price: CONSELEITE-RS March 2020.

RESULTS AND DISCUSSION

As expected, our results indicate that selective therapy significantly reduces the direct costs of treatments of clinical mastitis cases, since there were significant reductions of the number of treatments in costs with intramammary antibiotics, the volume of milk discarded, and demand for veterinary services. The results of the direct cost analysis with the treatments used during the study period are shown in figure 1.

Per[1]	**Trat**[2]	**Costs with antibiotics**	**Milk disposal costs**	**Volume of milk Discarded**	**Cost with veterinarian**	**Number of therapeutic protocols**	**Total cost per treatment**
P1a	T1	$ 16.73	$ 55.30	204.8 L	$ 1.55	95	$ 73.57

ISSN 1519 9940

RBSPA
Revista Brasileira de Saúde e Produção Animal
Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

P1b	T2	$ 11.10	$ 62.42	231.2 L	$ 1.55	266	$ 75.08
P2a	T3	$ 19.97	$ 54.62	202.3 L	$ 1.55	6	$ 76.14
	T4	$ 27.85	$ 62.42	231.2 L	$ 1.55	33	$ 91.82
	T5	$ 29.75	$ 62.42	231.2 L	$ 1.55	8	$ 93.72
P2b	T3	$ 19.97	$ 63.00	210 L	$ 1.55	6	$ 84.52
	T4	$ 27.85	$ 72.00	240 L	$ 1.55	103	$ 101.40
	T5	$ 29.75	$ 72.00	240 L	$ 1.55	17	$ 103.30

Figure 1 Direct cost estimated for each clinical case of mastitis, considering the different treatments used throughout the study period.

[1]: Periods included in the study: P1a - September to December 2017; P1b - January to September 2018; P2a -October 2018 to June 2019, P2b - July to October 2019.
[2]: Treatments used: T1 - Mastijet®Fort IMM 4 days; T2 - Spectramast®LC IMM 4 days; T3 - Ciprolac® IMM and 7.5 mg / Kg of enrofloxacin 10% IM for 3 days; T4 - Ciprolac® IMM for 5 days and 7.5 mg / Kg of enrofloxacin 10% IM for 3 days; T5 - Spectramast® IMM for 4 days, and 2 doses of 15 mg / Kg of Clamoxil® IM, with 48 hours of interval between applications

According to the results of our study, from September 2017 to October 2019, 599 cases of clinical mastitis were diagnosed, representing 39% of the 1500 animals analyzed. During P1, when selective therapy was not used, 95 cases of clinical mastitis occurred in P1a and 266 cases in P1b, totaling 361 cases. The average cost of clinical mastitis therapies per case was US74.33. The total cost of treatments, considering drugs, veterinary service, conventional culture, and milk disposal during period P1 corresponded to US27,559.97. Of this total, 79.3% (US21,857.90) corresponds to the disposal of milk (equivalent to 80,955.2 liters), 16.5% (US4,542.78) of the costs were attributed to the use of intramammary antibiotics, and 4.2% (US1159.29) to veterinary services and conventional culture.

During P2, when selective therapy was used, there was a 34,1% reduction in the number of cases of mastitis compared to P1, with 238 clinical mastitis diagnosed (123 cases less than in P1). From 238 cases, 165 cultures were performed to identify the etiological agent, 55 have occurred in P2a, with 45 cultures being performed and 183 in P2b, for which 120 cultures were performed. The total cost of treatments, considering medication, veterinary service (including the cost of plates in this service), and milk disposal during this period corresponded to US17,884.34. Of this total, 68.3% (US12,225.90) corresponded to the disposal of milk (equivalent to 40,753 liters), 24.9% (US4,449.69) of costs were attributed to the use of intramammary and injectable antibiotics, and 6.8% (US1,208.75), to veterinary services and plates. The average cost of of clinical mastitis therapies per case was US91.82.

In the conventional microbiological investigation performed during P1 (n = 157 cultures performed), in 85% (n = 134) of all samples, there was microbiological growth and the agent was identified. The pathogen most frequently detected was *Streptococcus uberis* (n = 22; 14%), followed by *Corynebacterium bovis* (n = 17; 11%) and *Streptococcus dysgalactiae* (n = 12; 8%). The cost of conventional microbiological culture per sample of US3.82, and a total cost of US599.74.

Considering the on-farm cultures performed in P2 (n = 165), in 64% (n = 105) of all samples, there was microbiological growth, and the agent was identified. The pathogen most frequently detected was *Streptococcus uberis* (n = 33; 20%), as well as in previous studies where the frequency of the agent was detected in 32% (McDougall et al., 2007) and 24.9% (Ganda et al., 2016) of the cultures performed. The second most frequent pathogen was *Streptococcus agalactiae* (n = 13; 8%), followed by *Streptococcus agalactiae / Dysgalactiae* (n = 12; 7%) and *Escherichia coli* (n = 8; 5%). The detailed distribution of P2 pathogens is presented in the Table 3. In 36% of cases of clinical mastitis, there was no growth of microorganisms, similar to that found in other studies that reported no growth of microorganisms in about 30% of cultures performed (Vasquez et al., 2016; McDougall et al., 2018), with no treatments being performed in these cases.

Table 3 Main causative agents of clinical mastitis from 165 samples of plate cultures of the farm during the period from October 2018 to October 2019.

INSULATED AGENTS	AMOUNT	%
No growth	60	36
Str. Uberis	33	20
Str.agalactiae	13	8
Str. Agalactiae / Str. Dysgalactiae	12	7
Escherichia coli	8	5
Enterobactersp	6	4
Other G + / G-	3	2
Str. Agalactiae / Sf. Aureus	4	2
Sf. Aureus	4	2
Str. Dysgalactiae	3	2
Sf. negative coagulase	3	2
Sf. aureus / Str. Uberis	2	1
Protohecassp	2	1
Enterococcus spp.	1	1
Other G-	1	1
Escherichia coli / Str. Dysgalactiae	1	1
Klebsiella / Enterobacter spp.	1	1
Yeast / Klebsiellasp	1	1
Str. Agalactiae / Str. Uberis	1	1
Str. Agalactiae / Enterobactersp	1	1
Str. Dysgalactiae / Klebsiellasp	1	1
Str. Dysgalactiae / Sf. Aureus	1	1
Str. Dysgalactiae / Sf. negative coagulase	1	1
Str. Uberis / Klebsiellasp	1	1
Sf. Haemoliticus	1	1
TOTAL	165	100

Streptococcus - Str. Staphylococcus - Sf.

According to the data analyzed regarding the therapeutic protocols used, during the study P1, out of 361 cases, 58 were (16%) relapses, and 61 were (16.8%) recurrences or new cases. When comparing the periods P1 and P2, there was a reduction of 2.6% in the frequency of relapses cases in P2 in relation to P1 (P1 = 16% and P2 = 13.4%) and of 1.3% in the frequency of recurrences cases (P1

Rev. Bras. Saúde Prod. Anim., Salvador, v.24, 01 - 11, 20230014, 2023
http://dx.doi.org/10.1590/S1519-994020230014

= 16.8% and P2 = 15.5%). The higher rates of recurrences found in P1 may indicate the presence of organisms resistant to antibiotics used in the protocols (P1 T1 / T2) and/or low clinical cure rates (Apparao et al., 2009). In P2, clinical cases of relapses were observed in 32 animals. However, in 9 of them, there was no microbial growth. In 2 cases, there was a growth of *Escherichia coli*, not requiring treatment. As for the recurrence of cases of the same period, out of 37 cases, 12 did not show microbial growth, which means that even with slight reduction between the two periods (1.3%), there was a significant reduction in the use of antibiotics and expenses with treatments of relapse cases in this period. These results suggest greater efficiency of treatments of P2 to those used in P1. The strategy of treating clinical mastitis cases only when microbiological growth in the microbial culture was observed, and in addition not treating clinical mastitis cases caused by *Escherichia coli* have reduced the antibiotics use and milk disposal in the present study (Roberson, 2003; Neeser, et al. 2006; Lago et al., 2011; Pinzón-Sánchez et al., 2011). This practice is supported by the results of previous studies (Leininger et al., 2003; Pyörälä et al., 1994; Fuenzalida & Ruegg, 2019), who compared treated and untreated clinical mastitis caused by *Escherichia coli* mastitis, showing very similar cure rates.

In our study, according to the results of the microbiological cultures (Table 4), in 68 cases, there was no growth of etiologic agent or growth of *Escherichia coli* (except for 3 cases that were treated for presenting grade 3 clinical mastitis). During the period of use of onfarm culture to identify the etiological agents of clinical mastitis in cows and the institution of selective treatments only in cows that show microbiological growth, there was a reduction of the disposal of 15,600 liters of milk, considering that the 65 untreated cases were treated with the gram-positive protocol. Emphasizing the importance of performing the culture in plates, if possible, associated with the isolation of agents and antibiogram, thus reducing the use of antibiotics, which could favor the appearance of agents resistant to the active principle used.

Considering the total number of clinical mastitis cases during P2, if conventional therapy was used, all diagnosed cases would have been treated (n = 238 cases). The cost would have been approximately US23,605.81 (considering adopting the T4P2a protocol for the 55 cases in this period and the T4P2b for the 183 of the second). However, with the use of onfarm culture and selective treatment of clinical mastitis, 65 fewer cases were treated (27.3%), that is, only 173 cases, totaling a cost of US17,884.34 (considering the cost with the veterinarian and the 265 plates), and savings estimated at US5,721.47. Still, considering the two years of the study, the estimated cost of treating clinical mastitis cases was US45,444.31, with US27,559.97 spent in P1 and US17,884.34 in P2. Based on the identification of mastitis pathogen types associated with clinical mastitis on the farm, selective therapy there was reduction of around 24.23%, or about US6,000.00 in the first year of implementation.

The adoption of selective treatment of clinical mastitis currently does not happen massively yet in Brazil, as many producers are unaware of the real economic impact of this methodology. This study suggests the economic and epidemiological advantages of using selective clinical mastitis treatment, which for the adoption this strategy by other farms. In the long term, this will reflect not only in reducing the economic impacts related to mastitis but also in using antibiotics more prudently.

CONCLUSIONS

In conclusion, the study demonstrates that the adoption of selective therapy as a method of treatment of clinical mastitis in herds of lactating cows presents advantages both in economic and epidemiological aspects, evidenced during the first year of implantation. Such benefits include a reduction in the use of antibiotics, a reduction in treatment costs, and a reduction in the volume of discarded milk.

Acknowledgments

Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Faculdade de Veterinária, Universidade Federal de Pelotas.

REFERENCES

APPARAO, M.D.; RUEGG, P.L.; LAGO, A.; GODDEN, S.; BEY, R.; LESLIE, K. Relationship between in vitro susceptibility test results and treatment outcomes for gram-positive mastitis pathogens following treatment with cephapirin sodium. **Journal of Dairy Science**, v.92, p.2589–2597, 2009.DOI: https://doi.org/10.3168/jds.2008-169t

FERREIRA, J.C.; GOMES, M.S.; BONSAGLIA, E.C.R.; CANISSO, I.F.; GARRETT, E.F.; STEWART, J.L.; ZHOU, Z.; LIMA, F.S.. Comparative analysis of four commercial on-farm culture methods to identify bacteria associated with clinical mastitis in dairy cattle. **PLOS ONE**, v.13, p.e0194211, 2018. DOI: https://doi.org/10.1371/journal.pone.0194211

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.5438-5457, 2019. DOI: https://doi.org/10.3168/jds.2018-16156

GANDA, E.K.; BISINOTTO, R.S.; DECTER, D.H.; BICALHO, R.C.. Evaluation of an On-Farm Culture System (Accumast) for Fast Identification of Milk Pathogens Associated with Clinical Mastitis in Dairy Cows. **PLOS ONE**, v.11, p.e0155314, 2016. DOI: https://doi.org/10.1371/journal.pone.0155314

GARCIA, S.N.; OSBURN, B.I.; CULLOR, J.S. A one health perspective on dairy production and dairy food safety. **One Health**, v.7, p.100086, 2019. DOI: https://doi.org/10.1016/j.onehlt.2019.100086

GONÇALVES, J.L.; KAMPHUIS, C.; VERNOOIJ, H.; ARAÚJO, J.R.J.P.; GRENFELL, R.C.; JULIANO, L.; ANDERSON, K.L.; HOGEVEEN, H.; DOS SANTOS, M.V. Pathogen effects on milk yield and composition in chronic subclinical mastitis in dairy cows. **The Veterinary Journal**, v.262, p.105473, 2020. DOI: https://doi.org/10.1016/j.tvjl.2020.105473

GUIMARÃES, J.L.B.; BRITO, M.A.V.P.; LANGE, C.C.; SILVA, M.R.; RIBEIRO, J.B.; MENDONÇA, L.C.; MENDONÇA, J.F.M.; SOUZA, G.N. 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. DOI:

https://doi.org/10.1016/j.prevetmed.2017.04.011

HE, W.; MA, S.; LEI, L.; HE, J.; LI, X.; TAO, J.; WANG, X.; SONG, S.; WANG, Y.; WANG, Y.; SHEN, J.; CAI, C.; WU, C. Prevalence, etiology, and economic impact of clinical mastitis on large dairy farms in China. **Veterinary Microbiology**, v.242, p.108570, 2020. DOI: https://doi.org/10.1016/j.vetmic.2019.108570

KUMAR, N.; MANIMARAN, A.; KUMARESAN, A.; SREELA, L.; PATBANDHA, T.K.; TIWARI, S.; CHANDRA, S. Episodes of clinical mastitis and its relationship with duration of treatment and seasonality in crossbred cows maintained in organized dairy farm. **Veterinary world**, v.9, p.75, 2016. DOI: 10.14202/vetworld.2016.75-79

LAGO, A.; GODDEN, S.M.; BEY, R.; RUEGG, P.L.; LESLIE, K. 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, 2011. DOI: https://doi.org/10.3168/jds.2010-4046

LEININGER, D.J.; ROBERSON, J.R.; ELVINGER, F.; WARD, D.; AKERS, R.M. Evaluation of frequent milk out for treatment of cows with experimentally induced Escherichia coli mastitis. **Journal of the American Veterinary Medical Association**, v.222, p.63–66, 2003. DOI: https://doi.org/10.2460/javma.2003.222.63

MANYI-LOH, C.; MAMPHWELI, S.; MEYER, E.; OKOH, A. Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. **Molecules**, v.23, p.795, 2018. DOI: https://doi.org/10.3390/molecules23040795

MCDOUGALL, S.; ARTHUR, D.; BRYAN, M.; VERMUNT, J.; WEIR, A.M. A 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. DOI: https://doi.org/10.1080/00480169.2007.36762

MCDOUGALL, S.; NIETHAMMER, J.; GRAHAM E. Antimicrobial usage and risk of retreatment for mild to moderate clinical mastitis cases on dairy farms following on-farm bacterial culture and selective therapy. **New Zealand Veterinary Journal**, v.66, p.98–107, 2018. DOI: https://doi.org/10.1080/00480169.2017.1416692

NEESER, N.L.; HUESTON, W.D.; GODDEN, S.M.; BEY, R.F. Evaluation of the use of an on-farm system for bacteriologic culture of milk from cows with low-grade mastitis. **Journal of the American Veterinary Medical Association**, v.228, p.254–260, 2006. DOI: https://doi.org/10.2460/javma.228.2.254

PINZÓN-SÁNCHEZ, C.; CABRERA, V.E.; RUEGG, P.L. 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. DOI: https://doi.org/10.3168/jds.2010-3930

PYÖRÄLÄ, S. Treatment of mastitis during lactation. **Irish Veterinary Journal**, v.62, p.40, 2009. DOI: 10.1186/2046-0481-62-S4-S40
Pyörälä, S.; Kaartinen, L.; Käck, H.; Rainio, V. 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. DOI: https://doi.org/10.3168/jds.S0022-0302(94)76973-3

ROBERSON, J.R. Establishing treatment protocols for clinical mastitis. **Veterinary Clinics of North America: Food Animal Practice**, v.19, p.223–234, 2003. DOI: https://doi.org/10.1016/S0749-0720(02)00071-3

ROYSTER, E.; GODDEN, S.; GOULART, D.; DAHLKE, A.; RAPNICKI, P.; TIMMERMAN, J. Evaluation of the Minnesota Easy Culture System II Bi-Plate and Tri-Plate for identification of common mastitis pathogens in milk. **Journal of Dairy Science**, v.97, p.3648–3659, 2014. DOI: https://doi.org/10.3168/jds.2013-7748

RUEGG, P.L. A 100-Year Review: Mastitis detection, management, and prevention. **Journal of Dairy Science**, v.100, p.10381–10397 2017. DOI: https://doi.org/10.3168/jds.2017-13023

SCHMENGER, A.; LEIMBACH, S.; WENTE, N.; ZHANG, Y.; BIGGS, A.M.; KROEMKER, V. Implementation of a targeted mastitis therapy concept using an on-farm rapid test: antimicrobial consumption, cure rates and compliance. **Veterinary Record**, v.187, p.401-401, 2020. DOI: https://doi.org/10.1136/vr.105674

SHIM, E.H.; SHANKS, R.D.; MORIN, D.E. Milk Loss and Treatment Costs Associated with Two Treatment Protocols for Clinical Mastitis in Dairy Cows. **Journal of Dairy Science**, v.87, p.2702–2708, 2004. DOI: https://doi.org/10.3168/jds.S0022-0302(04)73397-4

SUOJALA, L.; KAARTINEN, L.; PYÖRÄLÄ S. Treatment for bovine *Escherichia coli* mastitis - an evidence-based approach. **Journal of Veterinary Pharmacology and Therapeutics**, v.36, p.521–531, 2013. DOI: https://doi.org/10.1111/jvp.12057

VASQUEZ, A.K.; NYDAM, D.V.; CAPEL, M.B.; CEGLOWSKI, B.; RAUCH, B.J.; THOMAS, M.J.; TIKOFSKY, L.; WATTERS, R.D.; ZUIDHOF, S.; ZURAKOWSKI, M.J. Randomized noninferiority trial comparing 2 commercial intramammary antibiotics for the treatment of nonsevere clinical mastitis in dairy cows. **Journal of Dairy Science**, v.99, p.8267–8281, 2016. DOI: https://doi.org/10.3168/jds.2016-11258

VRIES, E.M.M.; LÜCKING, J.; WENTE, N.; ZINKE, C.; HOEDEMAKER, M.; KRÖMKER, V. Comparison of an evidence-based and a conventional mastitis therapy concept with regard to cure rates and antibiotic usage. **Milk Science International-Milchwissenschaft**, v.69, p.27-32, 2016. DOI: https://doi.org/10.25968/MSI.2016.6