



Treatment of non-severe mastitis in Brazilian dairy cows: a comparative study between amoxicillin-clavulanic acid and a conventional protocol

Kevin Graham Smith de Almeida¹ Chester Batista² Mariana de Mattos Brose¹
Monike Quirino³ André Gustavo Cabrera Dalto^{1*}

¹Sector de Grandes Ruminantes, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), 91540-000, Porto Alegre, RS, Brasil. E-mail: andre.dalto@ufrgs.br. *Corresponding author.

²Zoetis Animal Health, São Paulo, SP, Brasil.

³Núcleo de Ensino e Pesquisa em Produção Animal (NEPPA), Instituto Federal Catarinense (IFC), Araquari, SC, Brasil.

ABSTRACT: The association of amoxicillin and clavulanic acid has shown high efficacy to treat mastitis worldwide, also promoting a shorter milk disposal period compared to other antimicrobials. However, no previous investigations regarding its application to treat mastitis in Brazilian dairy cows were developed. This study evaluated the effectiveness of amoxicillin-clavulanic acid to treat mastitis in Brazilian dairy cows with mastitis, comparing it with a reference protocol treatment combination of tetracycline, neomycin and bacitracin. Holstein cows (n = 468) from three different dairy herds in Brazil were assigned to two groups: combination of tetracycline, neomycin and bacitracin (n = 178; positive control group) and amoxicillin-clavulanic acid protocol (n = 290). Before the treatment, milk samples were collected and cultivated in chromogenic media. After finishing the antimicrobial treatment (intramammary), milk samples were also collected and cultivated in chromogenic media. Results from microbiological analysis obtained before and after treatment were compared to determine the healing rate. Nine different microbiological agents were identified: eight of environmental origin and one of contagious origin; being eight gram-positive and one gram-negative bacteria. The positive control group and the treatment group showed similar healing rate (86.5% and 90.3%, respectively; $P > 0.05$). No differences were found between the groups for the healing rate, when the causing agent was considered. Therefore, it is possible to indicate the amoxicillin-clavulanic acid-based protocol to treat intramammary mastitis in Brazilian dairy cows, achieving great healing rates and providing a substantial reduction in milk disposal.

Key words: broad spectrum, dairy cow, intramammary antibiotic, milk disposal, tetracycline.

Tratamento de mastite não severa em vacas de leite no Brasil: um estudo comparativo entre o uso de amoxicilina associada ao ácido clavulânico e um protocolo tradicional

RESUMO: A associação de amoxicilina e ácido clavulânico tem mostrado alta eficácia para o tratamento de mastite em diversos países, além de promover um menor período de carência em comparação a outros antimicrobianos utilizados no tratamento da mastite. Apesar disso, estudos considerando a sua utilização para o tratamento de mastite bovina nos rebanhos leiteiros brasileiros ainda não foram realizados. Portanto, esse estudo teve o objetivo de avaliar a efetividade do uso de amoxicilina associada ao ácido clavulânico no tratamento da mastite bovina em rebanhos leiteiros do Brasil e compará-los com os resultados obtidos a partir do uso de um protocolo referência (contendo tetraciclina, neomicina e bacitracina). Um total de 468 vacas da raça Holandesa de três diferentes rebanhos brasileiros foi utilizado no estudo, sendo 178 animais designados para o grupo tetraciclina, neomicina e bacitracina (controle positivo) e 290 animais designados para o grupo amoxicilina-ácido clavulânico. Antes de iniciar o tratamento, amostras de leite foram coletadas e cultivadas em meio cromogênico. Após finalizar o tratamento com os antimicrobianos, por via intramamária, amostras de leite foram novamente coletadas e cultivadas em meio cromogênico. Os resultados das análises microbiológicas realizadas antes e após a administração foram comparados para calcular a taxa de cura dos tratamentos. Nove diferentes agentes foram identificados nas amostras de leite (oito de origem ambiental e um de origem contagiosa, sendo oito agentes gram-positivos e um gram-negativo). O grupo de vacas tratadas com amoxicilina-ácido clavulânico apresentou taxa de cura semelhante à taxa de cura dos animais do grupo controle positivo (90,3% vs. 86,5%; $P > 0.05$). A taxa de cura dos grupos foi semelhante também quando se considerou o agente etiológico. Portanto, é possível indicar a associação de amoxicilina com ácido clavulânico no tratamento da mastite bovina em rebanhos brasileiros, atingindo boas taxas de cura e possibilitando expressiva redução no período de descarte do leite.

Palavras-chave: amplo espectro, antibiótico intramamário, bovinos de leite, descarte de leite, tetraciclina.

INTRODUCTION

Bovine mastitis is among the main diseases in dairy production, being the most common and biggest cause of loss to producers (RUEGG, 2003), mainly due to economic impairment as a consequence of milk disposal, followed by treatment

costs (DEMEU et al., 2015). It estimated that milk disposal and treatment costs represent up to 45% of the expenses related to mastitis occurrence in a dairy farm (DOEHRING & SUNDRUM, 2019). In addition, mastitis is responsible for the highest percentage of antimicrobial use in dairy herds, either to treat clinical cases or to prevent cases in the next

lactation. (ERSKINE et al., 2002; STEVENS et al., 2016; DOEHRING & SUNDRUM, 2019).

Currently, mastitis treatment using antimicrobials is performed via intramammary or systemic, and eight groups of antimicrobials, composed of more than 30 different active drugs, are available to be used. Despite the large number of available protocols to treat bovine mastitis, a challenge still faced in this context is the long withdrawal periods for some antimicrobials (DOEHRING & SUNDRUM, 2019), which increases milk disposal. Furthermore, it is important to consider the resistance of some strains' generation since the microorganisms reported to be related to the mammary gland and responsible for mastitis have different characteristics of virulence, pathogenesis and possibly resistance (WATTS, 1988; RUEGG, 2013).

In this scenario, the use of products containing amoxicillin and clavulanic acid was indicated as a potential alternative to counteract some limitations regarding bovine mastitis control. Amoxicillin is a member of the penicillin group of antibiotics that interferes with bacterial cell wall synthesis. Its association with clavulanic acid extends the spectrum of activity against penicillinase-producing bacteria since clavulanic acid provides a beta-lactamase inhibitory action (CASTLE et al., 2007). Furthermore, the association allows reducing amoxicillin levels, enabling a low residual load and; consequently, shortening the milk disposal period compared to other antimicrobials used for mastitis treatment (GRUET et al., 2001). This combination has shown high efficacy for several bacterial agents causing mastitis worldwide (KLIMAITE, 2003; PODPEČAN et al., 2004; VASIL' et al., 2009).

However, despite all the advantages related to the amoxicillin and clavulanic acid association, to our knowledge no previous investigations regarding its application to treat mastitis in Brazilian dairy cows were developed. Therefore, this study verified the therapeutic effectiveness of an amoxicillin-clavulanic acid-based protocol to treat Brazilian dairy cows with non-severe mastitis, comparing it with a positive control (a combination of tetracycline, neomycin, and bacitracin).

MATERIALS AND METHODS

The experiment was carried out between November 2019 and June 2020 in three Brazilian dairy farms, in the states of Minas Gerais, Paraná, and Rio Grande do Sul, with herds of 460, 800, and 750

dairy cows and an average production of 32, 38 and 34 liters/cow/day, respectively.

Animals' selection

Holstein cows presenting non-severe mastitis (absence of systemic clinical signs but alterations in the foremilk, i.e., presence of blood, flake or clots, or watery appearance, in the foremilk, with presence or absence of redness or heat in the udder) were used. The animals were selected according to the following criteria: good general health status, at least 3 functional breast quarters, no clinical mastitis in the previous 30 days, no history of chronic mastitis, 1-4 lactation cycles, and average milk production for the period > 25 liters/day. On the same day cows were selected, milk sample was aseptically collected from each animal for microbiological culture in plates containing a chromogenic culture media (AccuMast Plus[®]; FERA Animal Health).

The plating and reading procedures were performed according to the manufacturer's recommendations, and cows with only one infected mammary quarter and presenting only one bacterial strain per mammary quarter were selected, excluding those animals with acute mastitis (table 1). Furthermore, animals infected with *Staphylococcus aureus* were also not selected, since the farms did not apply the same procedures and protocols for these cases.

Experimental design, treatment, and efficiency evaluation

The 468 selected cows were assigned by convenience into two groups: treated with a combination of tetracycline, neomycin, and bacitracin ($n = 178$; positive control group) or with amoxicillin-clavulanic acid ($n = 290$). The treatments started on the same day the animals were diagnosed with clinical mastitis (after the first milk collection), and the antimicrobials were daily administered.

For the positive control, the Mastijet Forte[®] was used (Tetracycline Hydrochloride 200 mg, Neomycin Base 250 mg – equivalent to 365 mg of Neomycin Sulfate, Zinc Bacitracin 2,000 IU – equivalent to 28 mg, and Prednisolone 10 mg; MSD Saúde Animal). The amoxicillin-clavulanic acid treatment was performed using the Synulox[®] LC (Amoxicillin – Trihydrate – 200 mg, Clavulanic Acid – Potassium Clavulanate – 50 mg, and Prednisolone 10 mg; Zoetis).

Treatment length ranged from 3 to 7 days (table 1), according to the bacterial agent. Fourteen days after starting the treatment, a new collection of the

Table 1 - Bacteriological cure rate in Brazilian dairy cows treated for mastitis using either a combination of tetracycline, neomycin, and bacitracin or amoxicillin-clavulanic acid according to the microbiological agent identified.

Agent	Total of samples	Tetracycline, neomycin and bacitracin (n = 178)			Amoxicillin-clavulanic acid (n = 290)			P-value
		Pre-treatment	Post-treatment	BCR (%)	Pre-treatment	Post-treatment	BCR (%)	
		Positive samples	Negative samples		Positive samples	Negative samples		
<i>Aerococcus viridans</i> ^B	26	12	11	91.67	14	13	92.86	0.91
<i>Enterobacter</i> spp. ^A	12	5	4	80	7	7	100	0.37
<i>Enterococcus</i> spp. ^A	22	11	9	81.82	11	10	90.38	0.56
<i>Lactococcus lactis</i> ^C	27	11	9	81.82	16	14	87.5	0.70
<i>Coagulase-negative Staphylococci</i> ^A	126	45	41	91.11	81	75	92.59	0.77
<i>Staphylococcus haemolyticus</i> ^A	34	15	11	73.33	19	17	89.47	0.23
<i>Streptococcus agalactiae</i> ^A	95	33	31	93.94	62	52	83.87	0.11
<i>Streptococcus dysgalactiae</i> ^B	80	28	24	85.71	52	47	90.38	0.53
<i>Streptococcus uberis</i> ^C	46	18	14	77.78	28	27	96.43	0.10

BCR: bacteriological cure rate, ^A three days of treatment, ^B five days of treatment, ^C seven days of treatment.

milk and microbiological culture were performed, as previously described. Animals whose milk samples did not show bacterial growth of the same specie detected in the first collection were considered healed, confirming the treatment efficiency, and the bacteriological cure rate was calculated for each treatment.

Statistical analysis

Data were analyzed using R Studio (v. 4.3.1). The bacteriological cure rates of treatments according to the microbiological agent identified were compared using the Chi-square test. The relative risk (RR) was determined to establish the differences of healing between both treatments. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

On the first milk collection and microbiological culture, nine different bacteriological agents were identified (eight gram-positive and only one gram-negative: *Enterobacter* spp.). Considering the contamination origin, only one agent was identified as contagious (*Streptococcus agalactiae*). The number of cases according to the agents reported and treatment groups is described in figure 1. From all the cows treated in this study (468), a total of 416 animals presented milk samples in which the bacterial growth after treatment was not related to the same agent identified in the first analysis (treatment onset). Therefore, 416 out of 468 cows were considered healed from mastitis after being treated, regardless of the antimicrobial used (healing rate of 88.9%; table 1).

Considering each treatment, it was observed that in the positive control group 154 out of 178 cows were classified as healed (healing rate of 86.5%), while for the amoxicillin-clavulanic acid-based treatment, the healing rate was 90.3% (262/290). The RR value was > 1.0 (1.4); however, it was not statistically significant (95% confidence interval = 0.84 – 2.33; $P = 0.23$). The healing rates according to the bacteriological agent were not different between treatments (table 1).

DOEHRING & SUNDRUM (2019) described treatment efficiency and milk disposal period as two limitations for controlling bovine mastitis. The use of a product containing amoxicillin and clavulanic acid could be used as a potential alternative to counteract these limitations in the control of bovine mastitis. In our study, we assessed the therapeutic effectiveness of this association to treat Brazilian dairy cows with mastitis, comparing it with a reference intramammary antibiotic containing tetracycline, neomycin and bacitracin. To our knowledge, this is the first investigation concerning the use and effectiveness of amoxicillin-clavulanic acid association to treat mastitis in Brazilian herds, and our results showed that both treatments had an overall bacteriological cure rate $> 86\%$, indicating that amoxicillin-clavulanic acid is efficient to treat bovine mastitis in Brazilian herds, similarly to studies performed worldwide (KLIMAITĚ, 2003; PODPEČAN et al., 2004; VASIL' et al., 2009), and reinforcing the feasibility of tetracycline for the same purpose.

Unsurprisingly, the medicine used as a positive control in our methodology was an intramammary antimicrobial formulated with the combination of tetracycline, neomycin, and bacitracin,

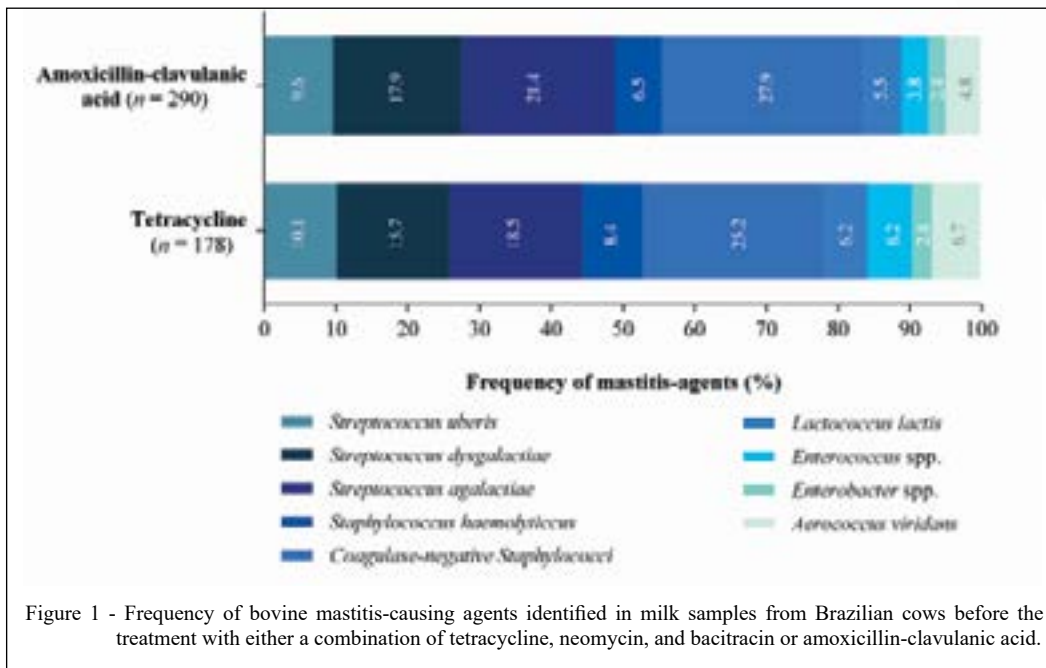


Figure 1 - Frequency of bovine mastitis-causing agents identified in milk samples from Brazilian cows before the treatment with either a combination of tetracycline, neomycin, and bacitracin or amoxicillin-clavulanic acid.

one of the most common drugs to treat bovine mastitis. TOMAZI & SANTOS (2020) recently demonstrated that intramammary drugs for treating mastitis composed of tetracycline, aminoglycoside, and polypeptide had the highest frequency of use in dairy herds in Brazil, which is due to their known effectiveness. In *in vitro* tests, about 96% of streptococcus isolates were susceptible to tetracycline (CONSTABLE et al., 2017), and its association with neomycin, and bacitracin has already been used as a positive control group in other studies to assess alternative treatments to bovine mastitis (CORTINHAS et al., 2016). The direct comparison between two products with the same purpose facilitates choosing the most advantageous treatment in the practice routine.

The ideal management in cases of bovine mastitis is to first perform microbiological culture for identifying the causative agent and then establish the most adequate protocol treatment (LAGO et al., 2011; ROBERSON, 2012). Most of the bacteria involved in the mastitis cases of this experiment were gram-positive. In cases of gram-positive bacteria, intramammary treatment is always recommended, except for those caused by *Staphylococcus aureus*, in which a critical evaluation case-by-case is strongly suggested, considering the possibility of discarding the animal (LAGO et al., 2011; ROBERSON, 2012). Our data also showed the occurrence of positive samples for gram-negative bacteria: *Enterobacter spp.* These cases were treated either with amoxicillin-clavulanic acid or with a combination of tetracycline,

neomycin, and bacitracin to assess the amoxicillin-clavulanic acid efficiency in Brazilian herds. However, it is noteworthy that using antimicrobials to treat mastitis is not a practical recommendation when the cause involves gram-negative bacteria (PINZÓN-SÁNCHEZ et al., 2011).

For some authors, the optimal protocol to treat bovine mastitis is the one providing the best possible cure in conjunction with the shortest possible withdrawal period (CRAVEN, 1987; SHIM et al., 2004). However, due to economic considerations, the costs directly related to antibiotic acquisition are also fundamental. Indeed, amoxicillin-clavulanic acid can be considered a costly product. Some years ago, despite the association having been widely used for treating mastitis in Chinese dairy herds with good clinical efficacy, its application was subjected to great restrictions because of its expensive price (LI et al., 2014). For this reason, it is strongly necessary to consider the treatment cost-benefit, since the product is efficient and promotes a quite short milk disposal period, decreasing the costs with the milk withdrawal. According to the manufacturers, the intramammary antimicrobial used in our methodology required a disposal period of nine days, while the alternative protocol required only three days of disposal. Considering an average daily milk production of 25 liters (the minimum value considered to select the cows in this study), it is estimated that shortening the milk disposal period from nine to three days would allow the producer to

avoid the discard of 150 liters of milk.

Overall, our results are useful to set the efficiency of this antibiotic association to treat mastitis considering the herds used and the bacterial agents found in our study. This alternative treatment must be considered by veterinarians and farmers when establishing mastitis treatment in Brazil, mainly taking into account the need for high efficiency and a short milk disposal period simultaneously. Moreover, this investigation can supply preliminary data for new approaches, since further studies focusing on the effectiveness of amoxicillin acid clavulanate in dairy herds are still needed, mainly considering herds from other Brazilian regions, a greater number of animals, different bacteriological agents, comparison with other drugs, as well as the comparison to a non-treated group.

CONCLUSION

In conclusion, amoxicillin-clavulanic acid presented similar healing rates compared to the combination of tetracycline, neomycin, and bacitracin, being a feasible alternative to treat mastitis in Brazilian dairy herds, providing shorter milk disposal and; consequently, decreasing the related expenses.

ACKNOWLEDGMENTS

We are thankful to Zoetis Animal Health for the support, to the Universidade Federal do Rio Grande do Sul (UFRGS) for the scholarship provided. The study was partially funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil - Financial Code 001, INCT Project 406866/2022-8 from the National Council for Scientific and Technological Development (CNPq), and projects No. 22/2551-0000558-6 and No. 23/2551-0000904-8 from the Research Support Foundation of the State of RS (FAPERGS).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

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

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The experimental procedure followed the Brazilian Federal Law of Animal Protection and was approved by the Animal Experiments Committee of Universidade Federal do Rio Grande

do Sul under number 34162.

REFERENCES

- CASTLE, S. S. Amoxicillin. In ENNA, S.J; BYLUND, B.B. **XPharm: The Comprehensive Pharmacology Reference**. Elsevier, 2007. p.1-6
- CONSTABLE, P. D. et al. Veterinary Medicine — A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, 11th edition. **Canadian Veterinary Journal**, v.51. 541 p.2017.
- CORTINHAS, C. S. et al. Randomized clinical trial comparing ceftiofur hydrochloride with a positive control protocol for intramammary treatment of nonsevere clinical mastitis in dairy cows. **Journal of Dairy science**, v.99, n.7, p.5619-5628, 2016. Available from: <<https://doi.org/10.3168/jds.2016-10891>>. Accessed: May, 24, 2023. doi: 10.3168/jds.2016-10891.
- CRAVEN, N. Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation—a review. **British Veterinary Journal**, v.143, n.5, p.410-422, 1987. Available from: <[https://doi.org/10.1016/0007-1935\(87\)90018-2](https://doi.org/10.1016/0007-1935(87)90018-2)>. Accessed: Jun. 02, 2023. doi: 10.1016/0007-1935(87)90018-2.
- DEMEU, F. A. et al. Influência da escala de produção no impacto econômico da mastite em rebanhos bovinos leiteiros. **Revista Ceres**, v.62, n.2, p.167–174, 2015. Available from: <<https://doi.org/10.1590/0034-737X201562020006>>. Accessed: May, 18, 2023. doi: 10.1590/0034-737X201562020006.
- DOEHRING, C.; SUNDRUM, A. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level. **Preventive Veterinary Medicine**, v.165, n.February 2018, p.63–70, 2019. Available from: <<https://pubmed.ncbi.nlm.nih.gov/30851929/>>. Accessed: Jun. 03, 2023. doi: 10.1016/j.prevetmed.2019.02.004.
- ERSKINE, R. J. et al. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. **Journal of Dairy Science**, v.85, n.5, p.1111–1118, 2002. Available from: <[https://doi.org/10.3168/jds.S0022-0302\(02\)74172-6](https://doi.org/10.3168/jds.S0022-0302(02)74172-6)>. Accessed: May, 19, 2023. doi: 10.3168/jds.S0022-0302(02)74172-6.
- GRUET, P. et al. Bovine mastitis and intramammary drug delivery: review and perspectives. **Advanced Drug Delivery Reviews**, v.50, n.3, p.245-259, 2001. Available from: <[https://doi.org/10.1016/S0169-409X\(01\)00160-0](https://doi.org/10.1016/S0169-409X(01)00160-0)>. Accessed: Jun. 18, 2023. doi: 10.1016/S0169-409X(01)00160-0.
- KLIMAITĖ, J. Anti-mastitis medication ‘Synolux’, Mamexine, Mastimix and Lincomycin-F efficacy in treating bovine sub-clinical mastitis. In: Farm animal reproduction: Conserving local genetic resources, 2003. **Proceedings of Farm animal reproduction: Conserving local genetic resources**. Lithuanian Veterinary Academy, Kaunas, Lithuania, 2003, p.28.
- 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, n.9, p.4441–4456, 2011. Available from: <<https://doi.org/10.3168/jds.2010-4046>>. Accessed: May, 18, 2023. doi: 10.3168/jds.2010-4046.
- LI, H. et al. Pharmacokinetics and pharmacodynamics of a novel amoxicillin/sulbactam/prednisolone intramammary infusion in lactating cows after repeated administrations. **Veterinárni Medicína**, v.59, n.5, 2014. Available from: <<https://www.agriculturejournals.cz/pdfs/vet/2014/05/01>>.

- pdf>. Accessed: Jun, 05, 2023. doi: 10.17221/7515-VETMED.
- PINZÓN-SÁNCHEZ, C. et al. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. **Journal of Dairy Science**, v.94, n.4, p.1873-1892, 2011. Available from: <<https://doi.org/10.3168/jds.2010-3930>>. Accessed: May, 12, 2023. doi: 10.3168/jds.2010-3930.
- PODPEČAN, O. et al. Treatment of subclinical staphylococcal mastitis. **Slovenian Veterinary Research**, v.41, p.31, 2004. Available from: <<http://www.dlib.si/?URN=URN:NBN:SI:DOC-411X5NY0>>. Accessed: Jun. 02, 2023.
- ROBERSON, J. R. Treatment of Clinical Mastitis. **Veterinary Clinics of North America - Food Animal Practice**, v.28, n.2, p.271-288, 2012. Available from: <<https://pubmed.ncbi.nlm.nih.gov/22664208/>>. Accessed: Jun. 02, 2023. doi: 10.1016/j.cvfa.2012.03.011.
- RUEGG, P. L. Investigation of mastitis problems on farms. **Veterinary Clinics of North America - Food Animal Practice**, v.19, n.1, p.47-73, 2003. Available from: <<https://pubmed.ncbi.nlm.nih.gov/12682935/>>. Accessed: May, 18, 2023. doi: 10.1016/S0749-0720(02)00078-6.
- RUEGG, P. L. Antibiotic treatments for bovine mastitis: Who, what, when, how and why?. In: American Association of Bovine Practitioners Conference. **Proceedings of American Association of Bovine Practitioners**. 2013. p.72-78.
- SHIM, E. H. et al. Milk loss and treatment costs associated with two treatment protocols for clinical mastitis in dairy cows. **Journal of Dairy Science**, v.87, n.8, p.2702-2708, 2004. Available from: <[https://doi.org/10.3168/jds.S0022-0302\(04\)73397-4](https://doi.org/10.3168/jds.S0022-0302(04)73397-4)>. Accessed: Jun. 25, 2023. doi: 10.3168/jds.S0022-0302(04)73397-4.
- STEVENS, M. et al. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. **Journal of Dairy Science**, v.99, n.3, p.2118-2130, 2016. Available from: <<https://doi.org/10.3168/jds.2015-10199>>. Accessed: May, 25, 2023. doi: 10.3168/jds.2015-10199.
- TOMAZI, T.; SANTOS, M. V. Antimicrobial use for treatment of clinical mastitis in dairy herds from Brazil and its association with herd-level descriptors. **Preventive Veterinary Medicine**, v.176, p.104937, 2020. Available from: <<https://doi.org/10.1016/j.prevetmed.2020.104937>>. Accessed: May, 28, 2023. doi: 10.1016/j.prevetmed.2020.104937.
- VASIL, M. Etiology, course and reduction of incidence of environmental mastitis in the herd of dairy cows. **Slovak Journal of Animal Science**, v.42, n.3, p.136-144, 2009. Available from: <http://www.vuzv.sk/slju/09_3/Vasil.pdf>. Accessed: May, 22, 2023.
- WATTS, J. L. Etiological Agents of Bovine Mastitis. **Veterinary Microbiology**, v.16, p.41-66, 1988. Available from: <<https://pubmed.ncbi.nlm.nih.gov/3354192/>>. Accessed: May, 22, 2023.