Ciência Rural, Santa Maria, v.52:1, e20200893, 2022 http://doi.org/10.1590/0103-8478cr20200893
ISSNe 1678-4596
MICROBIOLOGY

Training, development, and evaluation of on-farm culturing as a technical resource to reduce the use of antimicrobials in dairy herds in the Midwest Region of Santa Catarina State, Brazil

Caroline Auer[1] Ana Flavia Begnini[1] Leonardo Leite Cardozo[2] Eliete Griebeler[1] Diogenes Dezen[1] Deise Aline Knob[3] Marcella Zampoli Troncarelli[1*]

[1]Laboratório de Microbiologia Veterinária, Instituto Federal Catarinense, Campus Concórdia, 9703-720, Concórdia, SC, Brasil. E-mail: marcella.troncarelli@ifc.edu.br. *Corresponding author.
[2]Ordemilk Ltda, Treze Tílias, SC, Brasil.
[3]Universidade do Estado de Santa Catarina (UDESC/CAV), Lages, SC, Brasil.

ABSTRACT: *On-farm culturing is a technology booming in Brazil. It is based on the microbiological diagnosis of milk samples collected from clinical cases of bovine mastitis, on the farm where the animals are handled, quickly, simply, and at an affordable cost. With this resource, after 24 h of incubation, the isolated microorganisms are classified as gram positive or gram negative, helping to make decisions regarding the therapy of the animals. Considering the relevance of the Midwestern region of Santa Catarina State in the national dairy production scenario, the objectives of the study were: 1) to promote the technical training of the farmers in three dairy farms located in Treze Tílias-SC, 2) to assist in the implementation and evaluation of on-farm culturing as technological resource viewing the reduced use of antimicrobials in herds. In addition to the rapid acceptance by the farmers and their efficient training, with the use of the technology, there was a 45–50% reduction in the use of antimicrobials in dairy herds. This result demonstrated a significant effect on animal health, and determines an important cost reduction for farmers, as presented in detail in this manuscript.*
Key words: *bovine mastitis, diagnosis, treatment, laboratory.*

Capacitação, implementação e avaliação da cultura de leite na fazenda como recurso técnico voltado ao uso reduzido de antimicrobianos em rebanhos leiteiros no meio oeste catarinense

RESUMO: *A cultura de leite na fazenda (do inglês On farm culturing) é uma tecnologia em franca expansão no Brasil. Fundamenta-se no diagnóstico microbiológico de amostras de leite colhidas de casos clínicos de mastite bovina, na própria fazenda onde são manejados os animais, de forma rápida, simples e com custo acessível. Com este recurso, após 24 horas de incubação, os micro-organismos isolados são classificados em Gram-positivos ou Gram-negativos, auxiliando na tomada de decisões quanto à terapia dos animais. Considerando a relevância da região meio oeste catarinense no cenário nacional de produção leiteira, o objetivo do presente estudo foi promover a capacitação técnica dos produtores em três propriedades localizadas em Treze Tílias-SC, e auxiliar na implementação e avaliação da cultura de leite na fazenda como recurso tecnológico voltado ao uso reduzido de antimicrobianos nos rebanhos. Além da rápida aceitação pelos produtores e eficiente capacitação dos mesmos, verificou-se, com o emprego da tecnologia, redução de 45% a 50% do uso de antimicrobianos nos rebanhos. Este resultado, além de demonstrar significativo impacto em saúde animal, determinou importante redução de custos aos produtores, como apresentado detalhadamente no presente artigo.*
Palavras-chave: *mastite bovina, diagnóstico, tratamento, laboratório.*

INTRODUCTION

The exaggerated use of antimicrobials in farm animals is one of the main concerns nowadays owing to the effects on farm animals, the environment, and public health. In this context, we highlighted the artificial selection of strains of microorganisms resistant to antimicrobials, and the presence of residues of the active compounds and/or their metabolites in milk (RUEGG et al., 2017).

Especially in dairy cattle, the access to antimicrobials by farmers is somehow facilitated by the intense competition between the pharmaceutical industries, which makes the cost of these drugs quite accessible. Antimicrobials can be reported in wide distribution being commercialized, in large part, without prescription. Thus, the criterion for the treatment of animals is not always technically justified since in Brazil there are many farms without medical-veterinary assistance (VIEIRA et al., 2016).

Received 09.25.20 Approved 04.01.21 Returned by the author 06.01.21
CR-2020-0893.R1
Editors: Rudi Weiblen Juliana Felipetto Cargnelutti

One of the main diseases of dairy cattle that require intensive use of antimicrobials is clinical mastitis. This condition consists of an inflammation of the mammary gland, usually caused by bacterial microorganisms, resulting in a reduction in milk production, physical-chemical changes in milk, and local and/or systemic inflammatory signs (SANTOS & FONSECA, 2007; MEGID et al., 2016). Concerning the etiology of mastitis, TOMAZI et al. (2017) reported that 70% of the isolates from milk samples from clinical cases of bovine mastitis were represented by gram-positive bacteria and 27% gram-negative bacteria. These results corroborated those obtained at the Veterinary Microbiology Laboratory (LMV) of the Instituto Federal Catarinense (IFC), Concórdia campus. From February to June 2018, of 286 isolates from milk samples from clinical and subclinical mastitis cases, 119 (41.6%) were *Staphylococcus* spp., 88 (30.7%) *Streptococcus* spp., and 48 (16.7%) *Corynebacterium* spp., totaling 89% of gram-positive bacteria (unpublished data).

It is worth it to mention that mastitis prophylaxis, especially based on actions related to milking hygiene and adequate animal health management, must be the primary and central strategy to be adopted in dairy herds. From the economic point of view, this is much less expensive compared to the losses incurred by the treatment of clinical cases. Considering the high prevalence of contagious microorganisms in herds as already presented, its prevention is extremely important, particularly during milking procedures.

In another perspective, the occurrence of negative results for microbiological culture from milk samples is quite common. In the study by TOMAZI et al. (2017), it was reported that in 44% of cases of clinical mastitis there was no isolation of microorganisms for bacteriological culture. At the LMV of the IFC Concórdia campus, from February to June 2018, 500 milk samples collected from clinical and subclinical cases of bovine mastitis were analyzed; of these, 161 (32.2%) did not showed microbiological growth in the culture test. Although, some species of microorganisms are not isolated in conventional culture methods and/or are intermittently eliminated in milk, resulting in false-negative results for culture, the data presented reinforce the reports in the scientific literature that about 50% of cases of clinical mastitis are self-limiting; that means, they resolve themselves through the action of the immune system without the need of treatment (RUEGG, 2015).

Indeed, one of the critical points to be considered in the control of clinical cases of mastitis is the diagnosis. Generally, milkers consider the presence of lumps in milk as a determining factor to treat animals with antimicrobials. In a study carried out in Brazil, a total of 5,457 cases of clinical mastitis at the udder-quarter level (5,020 cases at the cow level) were registered; of these, 5,295 (97%) received antimicrobial therapy. The use of combined therapy (intramammary and injectable) was registered in 64.4% (n = 3,231) of the treatments (TOMAZI et al., 2017). These data reinforce the exaggerated and indiscriminate use of antimicrobials in herds.

Another aggravating factor is that when there are no established protocols for mastitis treatment, the criterion for the use of antimicrobials by farmers is generally based on economic issues, opting for lower-cost drugs, which are not always effective in eliminating infections. In this way, the possibility of bacteriological cure of the cow is reduced, leading to the chronicity of cases; the continued elimination of pathogens in the milk of infected cows acts as sources of infection for other animals in the herd. Additionally, the need to repeat the treatment, with a consequent increase in the therapeutic cost and pressure for the selection of genetically resistant bacterial strains, increases the number of days for discarding milk, and the partial and/or total loss of function of the affected mammary gland (s), determining the early culling of animals (VALMORBIDA et al., 2017).

To assist producers in making decisions for the targeted use of antimicrobials in clinical cases of mastitis in the dairy herd, researchers at the University of Wisconsin (Madison, USA) developed the technology called On-farm culturing. This is based on the microbiological diagnosis of clinical cases of bovine mastitis on the farm itself where the animals are managed quickly, simply, and at an affordable cost (MACEDO et al., 2013). With this feature, after 24h of incubation, microorganisms isolated from milk samples from clinical cases of mastitis are classified into gram-positive and gram-negative bacteria.

In cases where there is a therapeutic indication (isolation of gram-positive bacteria), it is possible to increase the cure rate since more efficient classes of antimicrobials are used against this group of bacteria. In contrast, when gram-negative bacteria are isolated (for mastitis cases grade 1 or 2), treatment with antimicrobials is not administered, as they are self-limiting processes, with the spontaneous cure of cows in 2/3 of the cases (RUEGG, 2015). Therefore, the unnecessary use of antimicrobials in animals is avoided, thus reducing the selection of genetically resistant bacterial strains, in addition to generating an important economic effect by reducing the total cost

of treatments. In studies carried out in the USA and Brazil, on dairy farms where on-farm culturing were instituted, there was a reduction in the order of 40–50% in the use of antimicrobials in herds (RUEGG, 2015).

Considering the relevance of the Midwestern region of the Santa Catarina state in the dairy agribusiness, characterized mainly by productive arrangements of family labor, the on-the-farm culturing was seen as an essential technology for these productive realities. Thus, the this study promoted the technical training of producers and assisted in the implementation and evaluation of farm culturing as a technological resource aiming to reduce the use of antimicrobials in dairy herds.

MATERIALS AND METHODS

Three dairy farms with predominantly black and white Holstein herd, from the municipality of Treze Tílias in the Midwest of Santa Catarina, were included in the study. All the farms presented the following pre-requisites: mechanized milking system; carrying out periodic individual milk somatic cell count (SCC), with a composite sample (all the daily milking), of lactating animals; identification and registration of cases of clinical mastitis; records of treatments performed in case of clinical mastitis; records of costs for treating clinical cases of mastitis; data management system; periodic veterinary medical assistance; economic conditions to enable the necessary infrastructure for the implementation/continuity of on-farm culturing on the dairy farm; and a person responsible for the laboratory.

During the study, farm "one' had approximately 100 lactating cows managed in a compost bedded pack barn system, with an average yield of 38 liters/cow/day; farm "two" had about 150 lactating cows managed in a free-stall system, consisting of an air-conditioning system of the wind tunnel type, with an average yield of 39 liters/cow/day~. Farm "three" had a herd of approximately 190 lactating cows managed in a free-stall system, with an average yield of approximately 40 liters/cow/day.

The three dairy farms started the technology on the farm, where they grew samples from clinical mastitis cases of grade 1 (lumps in milk) and 2 (lumps in milk associated with inflammation of the mammary gland). For reasons of animal welfare, from the first day of identification of the clinical mastitis (Grades 1 and 2), regardless of the laboratory result, treatment of the animal (s) affected was instituted with anti-inflammatory injection based on Meloxicam, every 24h for 3 days. Grade 3 mastitis (systemic signs) were treated immediately, with antimicrobials associated with anti-inflammatory and fluid therapy, if necessary.

Throughout the study, which was carried out between 2018 and 2019, periodic technical visits were made to the farms to train the farmers on the on-farm culturing, as well as guiding and monitoring the implementation of the laboratories. For this, lectures and practical classes in microbiological cultivation were given to the farmers in the respective laboratories of each farm. Besides, two "study days" were carried out on two farms involved in the study, to disseminate the technology to other farmers in the region, and raise their awareness of the importance of the rational use of antimicrobials in the herds.

The on-farm culturing technique was carried out as follows: to collect milk samples from grade 1 and 2 clinical mastitis cases, sterile Falcon type tubes (conical bottom) were used; the first jets of milk were discarded and the ends of the teats were adequately cleaned using cotton moistened with 2% iodized alcohol. The tubes were packed in plastic crates inside thermal boxes containing recyclable ice and immediately transported to the laboratories for processing.

For the quality control of the diagnoses, the samples were collected in duplicate, one of them was processed in the laboratory of the farm by the farmers, and the other one in the Laboratory of Veterinary Microbiology (LMV) of the Instituto Federal Catarinense (IFC), Concórdia campus by the technicians and teachers of the institute.

For the on-farm culturing, the samples were previously homogenized with the aid of sterile Swabians, the samples were inoculated on 5% bovine blood agar and MacConkey agar in a biplate using the striation by exhaustion technique (QUINN et al., 2005; MINNESOTA UNIVERSITY, 2016). Then, the plates were incubated at 37ºC in aerobiosis. After 18–24 h of cultivation, readings were performed and the results were compared with those obtained in the IFC LMV, which carried out the characterization of the microorganisms, using Gram staining and biochemical tests.

When gram-positive bacteria were isolated, the affected animal (s) were treated, using classes of drugs of the spectrum of action recommended for this purpose, according to the routine of each farm. In the case of isolation of gram-negative bacteria (cases of mastitis grades 1 and 2), treatment with antimicrobials was not carried out, and it was recommended that the milk from the affected udder quarter(s) be discarded for 3–5 days (PANTOJA, 2016). The bacteriological cure of these cases was evaluated through microbiological cultivation of milk

within 30 days after finding the clinical case/isolation of the gram-negative microorganism.

Additionally, as a way to assist in monitoring herds, microbiological cultivation of subclinical cases of mastitis were performed in cows with SCC higher than 200,000 cells/mL. The analyses were carried out every month, at the LMV of IFC campus Concórdia.

To assess the economic impact resulting from the reduction in the use of antimicrobials in cases of clinical bovine mastitis in the evaluated farms, the following data were collected: protocols and the average cost of the treatments used, average days of milk discharged, average daily yield of lactating animals, and average price received by the farmer per liter of milk.

RESULTS AND DISCUSSION

Regarding the training of the farmers, from October–December 2018, two lectures were held on each farm on the rationale, necessary infrastructure, and applications of the technology. The installation of the laboratory facilities was monitored, which was implemented in an average period of 1–3 months, according to the economic conditions of each farm. As soon as the laboratory facilities were completed, practical training in microbiological cultivation was given until the farmers were adequately prepared to carry out the procedures individually, and their results were consistent with those of the LMV.

Only two practical classes, within a week, were necessary for the farmers of the three farms to learn how to perform the technique properly, and to issue results consistent with those obtained in the IFC laboratory. The main challenge encountered by farmers at the beginning of the study was the aseptic collection of milk samples. The presence of contaminating microorganisms in the first samples evaluated by the farmers interfered with the agreement percentages with the results obtained by the IFC laboratory. However, the continued assistance of the team involved in the study allowed the farmers to be oriented in all stages of the process, and this problem was immediately remedied. Throughout the study, in farms 1, 2, and 3, the percentages of agreement with the results obtained by the LMV were 97.1%, 96.6%, and 94%, respectively. The great interest, motivation, and attention of the farmers and their assistants in this process are worthy of commendation.

As a way of complementary learning, two "study days" were held (in September and November 2019), for the dissemination of on-farm culturing technology and awareness of farmers regarding the rational use of antimicrobials in herds, in addition to other topics of interest in dairy cattle. The events were held at two farms involved in the study. The first had 45 participants, and the second 184 participants, including farmers, milkers, technicians, and other professionals in the dairy cattle industry. After participating in the events, many farmers were motivated to start the technology on their farms.

Considering the results of the on-farm culturing, in farm 1, 70 milk samples from clinical cases of mastitis were grown from March–October 2019. Of these, gram-positive bacteria were isolated in 38 (54.2%) and in 32 there was no need for treatment due to the absence of microbial growth (24 samples) or isolation of gram-negative (eight samples). There was a 45.8% decrease in the use of antimicrobials. On this farm, the average cost of treatment was R$ 449.00 for each case of clinical mastitis: R$ 50.00 for three intra-mammary tubes of the active ingredient ceftiofur hydrochloride and R$ 399.00 for 299 liters of milk that would be discarded during the 7-day grace period calculated from average production of 38 liters/cow/day at an average price of R$ 1.50 received per liter of milk. On this farm (1), there was a savings of R$ 1,700.00 with antimicrobials and R$ 13,566.00 with the non-disposal of milk, representing a total savings of R$ 15,266.00. It is worth mentioning that of the eight cases of clinical mastitis (grades 1 and 2) caused by gram-negative bacteria in which the treatment was not carried out, there was a bacteriological cure in seven (87.50%), indicating a self-limiting character of these conditions such as already reported in the literature (RUEGG, 2015). The animal that persisted with infection was at the end of lactation; therefore, the farmer chose to anticipate the drying of the cow and carried out prophylaxis therapy for the dry cow. After parturition, a colostrum sample was collected for microbiological cultivation and since microorganisms were not isolated, the cow was considered cured.

The approximate cost of installing the laboratory on farm 1 was R$ 7,000.00. The average cost per milk sample for analysis in private laboratories was R$ 34.00 (based on budgets provided by four laboratories located in the region where the study was conducted). With that, when carrying out the culturing of 70 milk samples on his farm, the farmer saved R$ 2,380.00. This amount added to the amount saved by the reduction in the use of antimicrobials making a total savings of R$ 17,646.00 during 8 months of evaluation. In this period, there was a return on investment and there was still a surplus of R$ 10,646.00.

In farm 2, between August and October 2019, 59 milk samples from clinical mastitis cases were cultivated, with gram-positive bacteria isolated in 29 (49.1%). In 30 cases there was no need for treatment, due to the absence of microbial isolation in the samples. Thus, the farm reduced the use of antimicrobials by 50%. On this farm, the average cost of treatment was R$ 459.50 for each case of clinical mastitis; R$ 50.00 for three intra-mammary tubes of the active ingredient ceftiofur hydrochloride and R$ 409.50 for 273 liters of milk that would be discarded during the 7-day grace period, calculated from average production of 39 liters/cow/day and an average price of R$ 1.50 received per liter of milk. There was a savings of R$ 1,500.00 with antimicrobials and R$ 12,285.00 with the non-disposal of milk, totaling R$ 13,785.00 in savings to the farmer. For the untreated cases, it was not possible to assess the bacteriological cure rate since there was no isolation of gram-negative bacteria.

The approximate cost of installing the laboratory on farm 2 was R$ 5,000.00. When cultivating the 59 samples on his farm, the producer saved R$ 2,006.00 (considering the average value of R $ 34.00 per milk sample for analysis in private laboratories). This amount added to the amount saved by the reduction in the use of antimicrobials, a total savings of R$ 15,791.00 during the 3 months of evaluation. Therefore, in this period there was a return on investment and there was still a surplus of R $ 10,791.00.

In farm 3, during September and October 2019, 50 milk samples were cultivated from clinical cases of mastitis, with the isolation of gram-positive microorganisms in 24 (48%). In 26 cases, there was no need for treatment (13 were due to the isolation of gram-negative bacteria and 13 were due to the absence of microbial growth in the samples). There was a 52% reduction in the use of antimicrobials. On this farm, the average cost of treatment was R$ 615.00 for each case of clinical mastitis, R$ 75.00 corresponding to three intra-mammary tubes of active ingredient that combines tetracycline hydrochloride, neomycin and bacitracin, and R$ 540.00 referring to 360 liters of milk that would be discarded in a 9-day grace period, calculated from the average production of 40 liters/cow/day and an average price of R$ 1.50 received per liter of milk. This farm saved R$ 1,950.00 with antimicrobials and R$ 14,040.00 with the non-disposal of milk, resulting in R$ 15,990.00 in total savings. It is also noteworthy that of the 13 cases of grade 1 or 2 mastitis identified with infection by gram-negative bacteria in which treatment was not instituted; there was a bacteriological cure in nine (70%). For the four cases in which the infection persisted, the farmer chose not to carry out the treatment, after evaluating the parity, history of SCC, and days in milk (DIM) of the animals. The period of 7 days grace period elapsed and microbiological culture of the milk samples were carried out, with no microbial isolation in the evaluated samples, and the cows were considered cured.

The approximate cost of implementing the laboratory on farm 3 was R$ 6,000.00. When cultivating the 50 samples on his farm, the farmer saved R$ 1,700.00 (considering the average value of R$ 34.00 per milk sample for analysis in private laboratories). This amount was added to the amount saved by the reduction in the use of antimicrobials, giving a total savings of R$ 17,690.00 during 2 months of evaluation. Thus, there was a return on investment and there was still a surplus of R$ 11,690.00.

Regarding the microbiological monitoring of subclinical cases of mastitis, performed by the LMV, a total of 1,377 milk samples were processed (676, 273, and 428 samples from farms 1, 2, and 3, respectively). There were microbial growth in 816 (59.25%) samples evaluated and in 485 (35.22%) there was no isolation of agents. In 76 (5.51%) samples it was not possible to make the diagnosis due to the presence of contaminating microorganisms. From the positive samples, 842 strains of microorganisms were isolated, with 792 (94.06%) gram-positive bacteria and 50 (5.95%) gram-negative bacteria.

Although, the majority of pathogens involved in the etiology of cases of clinical and subclinical mastitis in the herds evaluated were characterized by gram-positive bacteria, the technology of milk culture on the farm was still economically viable for farmers. The reduction in costs obtained in a few months due to the significant reduction in the use of antimicrobials and the absence of the need to dispose of milk, as shown, was sufficient to return the investment in the necessary laboratory infrastructure. The non treatment of some cows also reflected positively on animal health and welfare since the therapeutic approach always generates, to a greater or lesser degree, stress on the animals.

Regarding the absence of microbial isolation in the samples evaluated in the present study, it should be noted that this does not necessarily reflect the absence of infection since microorganisms such as *Mycoplasma* spp. are not detected by conventional microbiological methods. Similarly, to increase diagnostic accuracy against intracellular agents and/or intermittent elimination by milk, serial sampling and freezing/thawing of samples before cultivation

are necessary. Such conducts were not carried out in the present study, considering the particularities of milk culture on the farm, which requires isolation of agents in 18–24 h after cultivation, for decision-making regarding treatment (RUEGG et al., 2017).

The use of the culture media blood agar and MacConkey agar in biplates in the present study met the needs of the proposal; however, there are other types of media available on the market that can be used, aiming greater specificity in the characterization of the isolates. In place of blood agar, for example, the Factor medium can be used (UNIVERSITY OF MINNESOTA, 2016). In addition to the biplate, the triplate (additionally containing Edwards' or MTKT's medium for identification of streptococci and related species), quadriplate (additionally containing the Baird-Parker medium for identification of staphylococci), and chromogenic media (selective and differentials for several species of microorganisms) (GARCIA et al., 2019; BICALHO et al., 2020) can be used.

Besides the technical-operational and economic benefits obtained from the use of on-farm culturing, in the farms evaluated, it is important to highlight the social impact experienced in the present study since the farmers were very motivated with the training and "study days," reporting their greater appreciation and self-perception as dairy agribusiness entrepreneurs.

CONCLUSION

The results obtained in our study reinforce the benefits of on-farm culturing, especially under the economic, social, and animal health aspects. The practicality of this resource was demonstrated by the ease, speed, and accuracy with which the farmers operated the laboratory on the farm, after receiving training. Besides, the reduction of approximately 50% in the use of antimicrobials in the evaluated herds, in addition to positively impacting animal health and public health, consolidating the indication of this technology as an economically viable resource to be instituted in dairy farms in Brazil.

ACKNOWLEDGMENTS

The authors would like to thank the dairy farmers and milkers involved, for their interest and availability in collaborating with the research; to the staff of the Veterinary Microbiology Laboratory of the IFC campus Concórdia, for their assistance in laboratory analysis; To the IFC Pro-Rectors of Research, Teaching, and Extension for the granting of two scientific initiation grants to the students involved in the study and financial resources for the purchase of consumable materials charged to the research (Edital APL 130/18, Process number 4418/2019).

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was part of the "Pro Latte Program for mastitis prophylaxis and control", registered and approved with the CEUA of the IFC campus Concórdia under number 07/16.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The IFC Pro-Rectors of Research, Education, and Extension, as financiers of the scientific initiation scholarships, were not involved in the design of the study, nor the collection, analysis, or interpretation of the data; not even in the writing of the article or in the decision to publish the results.

AUTHORS CONTRIBUTION

All authors contributed equally to the writing of the article and revised it, approving the final version sent for publication.

REFERENCES

BICALHO, R. C. et al. Cultura microbiológica na fazenda para tratamento de mastite clínica reduz o uso de antibióticos sem afetar os índices produtivos. **Circular Técnica do Mestrado Profissional em Desenvolvimento Rural**, v.2, n.11, 2020. Available from: <https://home.unicruz.edu.br/wp-content/uploads/2020/12/CIRCULA-T%C3%89CNICA-V2-N11-CULTURA-MICROBIOL%C3%93GICA-NA-FAZENDA-PARA-TRATAMENTO-DE-MASTITE.pdf>. Accessed: Dec. 04, 2020.

GARCIA, B. L. N.; et al. Avaliação de meios de cultura cromogênicos para diagnóstico rápido de bactérias gram positivas causadoras de mastite subclínica. **XIII Simpósio de Pós-Graduação e Pesquisa em Nutrição e Produção Animal-VNP 2019**. Available from: <https://posvnp.org/novo/wp-content/uploads/2019/11/breno-luis-nery-garcia.pdf>. Accessed: Dec. 26, 2020.

MACEDO, S. N.; CORTINHAS, C. S.; SANTOS, M. V. Cultura microbiológica do leite na fazenda: uma nova ferramenta para o diagnóstico de mastite. **Revista Leite Integral**. Maio de 2013, p.6-12. Available from: <https://www.revistaleiteintegral.com.br/noticia/cultura-microbiologica-do-leite-na-fazenda-uma-nova-ferramenta-para-o-diagnostico-de-mastite>. Accessed: Nov. 28, 2020.

MEGID, J.; RIBEIRO, M. G.; PAES, A. C. **Doenças Infecciosas de Animais de Produção e de Companhia**. 1ª Ed. Rio de Janeiro: Roca, 2016. 1.294p.

MINNESOTA UNIVERSITY. **Minnesota Easy® Culture System User's Guide**. 2016. 29p. Available from: <https://www.vdl.umn.edu/sites/vdl.umn.edu/files/mn-easy-culture-system-ii-users-guide-english.pdf>. Accessed: Oct. 12, 2020.

PANTOJA, J. C. F. **I Curso de aperfeiçoamento em mastite bovina**. Tópicos avançados em diagnóstico, epidemiologia, tratamento, manejo preventivo e planos de controle – estudo de casos. Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu-SP. 28-30 de outubro de 2016.

QUINN, P. J. et al. **Veterinária e Doenças Infecciosas**. Ed. Artmed. Porto Alegre, 2005. 512p.

RUEGG, P. et al. On-Farm Culturing for Better Milk Quality. **Western Dairy Management Conference**. March, 2017, Reno, Nevada, USA, p.149-159, 2017. Available from: <http://wdmc.org/2009/On%20Farm%20Culturing%20for%20Better%20Milk%20Quality.pdf>. Accessed: Dec. 26, 2020.

RUEGG, P. **On-farm culture and treatment decisions**. 2015. Available from: <https://milkquality.wisc.edu/wp-content/uploads/sites/212/2015/06/Ruegg_On-Farm-Culture-and-Treatment-Decisions.pdf>. Accessed: Jan. 11, 2018.

SANTOS, M. V.; FONSECA, L. F. L. **Estratégias para controle de mastite e melhoria da qualidade do leite**. Manole: Pirassununga-SP, 2007. 314p.

TOMAZI, T.; et al. Associação entre características de rebanho e a frequência de uso de antimicrobianos para tratamento de mastite clínica. Departamento de Nutrição e Produção Animal; Universidade de São Paulo, Pirassununga, Brazil. **Anais do VII Congresso Brasileiro de Qualidade do Leite 28 e 29 de setembro de 2017**, Curitiba-PR, p.89-90.

UNIVERSITY OF MINNESOTA. **Minnesota Easy® Culture System User's Guide**, 2016. Available from: <https://www.vdl.umn.edu/sites/vdl.umn.edu/files/mn-easy-culture-system-ii-users-guide-english.pdf>. Accessed: Dec. 26, 2020.

VALMORBIDA, M. K. et al. Etiology and *in vitro* antimicrobial susceptibility profile of strains isolated from bovine mastitis in dairy herds from the Midwest region of Santa Catarina state, Brazil. **Acta Vet. Bras.**, v.11, p.219-225, 2017. Available from: <https://periodicos.ufersa.edu.br/index.php/acta/article/view/7266>. Accessed: Oct. 20, 2019. doi: 10.21708/avb.2017.11.4.7266.

VIEIRA, V. A. et al. Práticas de uso de antimicrobianos em rebanhos bovinos em 15 unidades de agricultura familiar no norte de Minas Gerais. **Cad. Ciênc. Agrá.**, v.8, n.1, p.08-15, 2016. Available from: <https://periodicos.ufmg.br/index.php/ccaufmg/article/view/2806>. Accessed: Sep. 15, 2019.