Impact of a Novel Homeopathic Complex Medicine on the Management of Multiple Antibiotic-Resistant Bovine Mastitis: An Open-Label, Non-Randomized, Placebo-Controlled Trial

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Homeopathy

Abstract

Background Bovine mastitis is characterized by an inflammatory process in the mammary gland and represents one of the main diseases affecting a dairy herd. Management of mastitis is most commonly via antibiotics, but the rising incidence of multiple antibiotic resistance (MAR) means that additional options are needed. Homeopathic products can be administered in dairy farming for a range of clinical reasons and may be preferential due to the absence of residues.

Objectives The aim of this study was to assess the potential of a novel homeopathic complex medicine in managing bovine mastitis.

Methods Twenty-four lactating Holstein cows with mastitis were divided into two groups: the homeopathic complex group received a homeopathic complex daily for 60 days at a dose of 20 g/d; the placebo group received the calcium carbonate vehicle without homeopathic medicines at the same dose and repetition. The main outcome measure was somatic cell count (SCC; cells/mL), with additional outcome measures including milk production (kg/d), milk constituents (percentage of protein, fat, lactose and total milk solids), and serum levels of cortisol, glucose, ammonia and lactic acid. All outcomes were measured at the beginning of the study and after 30 and 60 days. Milk samples were also collected from all animals at the beginning of the study, confirming a high ($>$0.2) MAR index for isolated bacterial cultures.

Results Assessment of SCC showed a statistically significant difference favoring the homeopathic complex versus placebo group at day 60. A reduction in serum cortisol levels and an increase in fat, lactose and total milk solids in animals treated with the homeopathic complex at day 60 were also seen. Other outcome measures did not show statistically significant inter-group differences.

Conclusion The results of this non-randomized, open-label, placebo-controlled trial suggest the potential for a novel homeopathic complex medicine in management of multiple antibiotic-resistant bovine mastitis, thus offering dairy farmers an additional option to antibiotics and making dairy products safer for consumer health and milk production more sustainable.

Keywords
- bovine mastitis
- homeopathy
- milk quality
- somatic cell count
- milk constituents

received
July 21, 2022
accepted after revision
February 22, 2023

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

DOI https://doi.org/10.1055/s-0043-1768465.
ISSN 1475-4916.

Introduction

Bovine mastitis is an inflammatory process of the mammary glands of cows, which impacts milk production and increases risk to the health of consumers,[1] especially when the milk is not subjected to adequate pasteurization,[2] because of the zoonotic toxin-producing microorganisms[1,3] that may be involved in the etiology of the condition.[4]

Antimicrobial drugs are traditionally used in the treatment of bovine mastitis; however, studies have shown an increasing rate of resistance of microorganisms to the antibiotics used, which leads to therapeutic failure, loss of milk quality, production losses, and impacts on cows' health.[5] In addition, the occurrence of residues of this pharmaceutical class in dairy products further encourages the emergence of bacterial resistance.[4]

The indiscriminate and inappropriate use of antibiotics has contributed to the acceleration of microbial resistance and selection of adapted microorganisms. Antibiotics increase selective pressure on bacterial populations, causing the death of susceptible bacteria and increasing the percentage of resistant bacteria. The propagation of antibiotic-resistant bacterial strains has caused significant challenges in dairy farming and in the treatment of diseases, causing economic damage to dairy production and the health of the herd.[6] Mastitis is the most common cause of antimicrobial use in dairy herds. Thus, bovine milk is considered a potential source of multidrug-resistant bacteria.[7] The rise in antibiotic-resistant bacteria has created an increasingly urgent need for alternative or complementary treatments.[8,9]

In this way, homeopathy emerges as a possible option for the treatment and prevention of bovine mastitis, potentially improving the quality of dairy production, ultimately being reflected in a safer product[10] with greater economic value.[11] The principles of the homeopathic treatment method are based on the "law of similars",[12] where substances are used that cause signs and symptoms similar to those of the ongoing condition, stimulating a secondary reaction of the organism against the disease.[13] Varied results are described in the scientific literature regarding the use of homeopathy in mastitis, related to different preparations, application routes and dosages.[14] To add to this evidence base, the present study aimed to evaluate the potential of a novel homeopathic complex medicine in the management of mastitis in multiple antibiotic-resistant cows.

Materials and Methods

Ethical Approval

The present research was veterinary in nature. All cows used were part of the Dairy Cattle Unit of the Universidade Paranaense; after completion of the study all animals returned to the breeding system according to the management of the farm. The study was approved by the Ethics Committee in Animal Science of Universidade Paranaense—protocol 25910 (**Supplementary file 1**, available online only). This committee is institutional but is governed and inspected by the National Council for the Control of Animal Experimentation, under the responsibility of the Ministry of Science, Technology and Innovation, at the national level in Brazil.

Herd Management

For the duration of the study, the cows were housed as usual in grassy paddocks. Good-quality feed for dairy cattle, clean water and mineral salt were available freely throughout the duration of the study. All animals were clinically examined periodically to ensure their on-going general health.

The somatic cell count (SCC) and the multiple antibiotic resistance (MAR) index were used to select the animals for inclusion in the study. Animals that presented with an SCC above 250,000 cells/mL of milk and an MAR index of over 0.2 were considered to have clinical mastitis.[15] Twenty-four lactating Holstein cows with mastitis were selected and divided into two groups with 12 animals each: one group called "homeopathic complex medicine" and the other "placebo". All selected cows were tested for antimicrobial resistance and confirmed to be multi-resistant to antibiotics prior to inclusion in the study.

Assignment to each group was not randomized. Instead, the cows were assigned so that there was uniformity in the mean SCC values between the two groups at baseline on day –2 before addition of any homeopathic or placebo supplements to the food.

Antimicrobial Resistance Testing

Antimicrobial susceptibility testing was performed as previously reported.[16] Briefly, each bacterial isolate from the milk samples per cow collected at baseline (day –2) was submitted to an antibiogram to determine its antimicrobial resistance. To assess antimicrobial susceptibility/resistance, the following antibiotics were used: amoxicillin + clavulanate (20/10 µg), ampicillin (10 µg), cephalexin (30 µg), cefoxetine (30 µg), ceftiofur (30 µg), clindamycin (2 µg), enrofloxacin (5 µg), streptomycin (300 µg), gentamicin (10µg), oxacillin (1 µg), penicillin (10 ui), sulfa + trimethoprim (23.75/1.25 µg).

The MAR index was calculated and interpreted according to Blasco et al[17] using the formula: a/(b.c), where "a" is the total number of antimicrobials to which the isolated bacteria showed resistance, "b" is the number of antimicrobials tested, and "c" is the number of isolates tested from the original sample. The index to delimit whether the samples are high or low risk was 0.200, where $\leq$0.199 is considered low risk and 0.200 and above is considered high risk.

Homeopathic Complex Medicine

The homeopathic complex medicine was specially designed for this study. The complex contained the following: *Asa foetida* 9cH, *Ignatia amara* 9cH, *Phytolacca decandra* 9cH, *Urtica urens* 6cH, *Pulsatilla nigricans* 6cH, *Calcarea carbonica* 9cH, *Sulphur* 9cH, *Lac vaccinum defloratum* 9cH, *Staphylococcus aureus* 9cH, and *Streptococcus* spp. 9cH. The original mother tincture (or a suitable intermediate potency) of each remedy was acquired from the laboratory of H&N Homeopatia e Produtos Naturais; the complex medicine was then prepared in the homeopathy laboratory of the company Minerphos by mixing the constituents, having brought them to the desired potencies according

to the Hahnemannian method.[18] A volume of 5 mL of each homeopathic medicine was added to 1 kg of calcium carbonate vehicle, giving a total volume of 50 mL homeopathic medicines per 1 kg of vehicle powder.

The homeopathic complex group received 20 g/d of the homeopathic complex powder added to individual food rations supplied daily after milking, for 60 days. Similarly, in parallel, the placebo group received 20 g/d of the calcium carbonate vehicle powder added to individual food rations supplied daily after milking, for 60 days. Treatment assignment was not blinded.

Somatic Cell Count

To assess the mean SCC per group, milk samples were collected from each individual cow at baseline on day −2, then on days 30 and 60. The milk samples from each cow were placed in polyethylene bottles with a screw cap and a capacity of 50 mL containing two tablets of the preservative bronopol (2-bromo-2-nitropropane-1, 3-diol), labeled and sent to the Laboratory of the Milk Analysis Program of the Association of Holstein Cattle Breeders in Curitiba, State of Paraná, using the Somacount 500 device (Bentley Instruments).

Milk Constituents

To assess the relative proportions of milk constituents, milk samples were collected in the same way as for the SCC assay in polyethylene bottles with preservative. Samples were assessed on day −2, day 30, and day 60 for percentage content of protein, fat, lactose and total milk solids. The analysis was performed using an infrared analyzer (Bentley 2000 equipment) and carried out in the dairy control laboratory of the Paraná Association of Holstein Cattle Breeders, located in Curitiba, Paraná.

Milk Production

To assess total milk production per animal at day 0, day 30 and day 60, the volume of milk collected during milking was measured manually using measuring cups and expressed in kg.

Serum Concentrations of Cortisol, Glucose, Ammonia, and Lactic Acid

To assess serum concentration of cortisol, glucose, ammonia and lactic acid, blood samples were taken by venipuncture of the caudal vein on day 0, 30, and 60. Blood samples were collected with 5 mL syringes and transferred into tubes without ethylenediaminetetraacetic acid. Blood samples were sent for analysis in the clinical analysis sector of Universidade Paranaense.

Statistical Analysis

Data were analyzed using the Bioestat 5.3 program using factorial analysis of variance for milk production data, SCC, milk composition, serum concentrations of cortisol, glucose, lactic acid and ammonia. For the MAR index, the Mann–Whitney test was used. Comparative analyses were performed between both groups. A two-tailed test was performed. A probability level of 5% was regarded as the threshold for statistical significance.[19]

Table 1 Mean ± standard error for somatic cell count (SCC) per milliliter of milk at days −2, 30 and 60 in cows treated daily with a homeopathic complex medicine or placebo.

Group	Time point (days)	SCC x 10^3/mL
Homeopathic complex	−2	325 ± 82.64
	30	257 ± 60.16
	60	246 ± 52.13
Placebo	−2	364 ± 96.74
	30	726 ± 239.57
	60	1,520 ± 524.04[a]

[a] $p < 0.05$ (placebo compared to homeopathic complex).

Results

Milk samples with MAR index rates greater than 0.2 can be considered at high risk of multi-resistance.[17] All cows included in the study were at high risk of multi-resistance: the cows in the homeopathic complex group had a mean MAR index of 0.385 and the placebo group had a mean MAR index of 0.393, with no significant difference between groups. The bacterial strains isolated from the milk of the animals in the present study showed resistance to the following antibiotics: ampicillin (10 µg), cephalexin (30 µg), ceftiofur (30 µg), streptomycin (300 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin (10 ui), and sulfa + trimethoprim (23.75/1.25 µg).

The results for SCC are shown in ►**Table 1**. A significant difference in SCC was observed between the groups at 60 days of the experiment ($p < 0.05$). The SCC remained relatively stable for the duration of the study in the homeopathic complex group but increased in the placebo group, indicating a potential improvement in immunological control of mastitis under homeopathic treatment.

An influence of the treatment with the homeopathic complex on the composition of the milk produced was also seen. ►**Table 2** shows the results of milk production (kg/d) and percentage of fat, percentage of protein, percentage of lactose and total solids of dairy cows treated with the homeopathic complex and the placebo groups. No differences were seen between groups at day 30, but significant differences ($p < 0.05$) were observed in the percentages of fat, percentages of lactose and percentage of total solids between the groups at day 60.

The use of the homeopathic complex also resulted in lower serum cortisol concentrations compared to the placebo group at day 60 (►**Table 3**), but not at day 30. Regarding plasma levels of lactic acid and ammonia, statistically significant differences were not observed between groups at any time point (►**Table 4**).

Discussion

The use of homeopathic products in the dairy herd has been tested in a number of studies and has been found to provide

Table 2 Mean ± standard error for milk production, percentage of fat, protein, lactose and total solids as constituents of milk taken at baseline, days 30 and 60 from cows treated daily with a homeopathic complex or placebo.

Group	Time point (days)	Milk production (kg/d)	Fat (%)	Protein (%)	Lactose (%)	Total solids (%)
Homeopathic complex	Baseline[b]	16.17 ± 0.15	3.67 ± 0.12	3.35 ± 0.13	4.28 ± 0.08	12.33 ± 0.25
	30	15.00 ± 0.16	3.73 ± 0.14	3.46 ± 0.11	4.41 ± 0.06	12.59 ± 0.20
	60	15.67 ± 0.17	3.99 ± 0.18	3.52 ± 0.13	4.46 ± 0.05	12.95 ± 0.27
Placebo	Baseline[b]	17.71 ± 1.23	3.37 ± 0.13	3.18 ± 0.09	4.14 ± 0.05	11.64 ± 0.26
	30	17.18 ± 1.4	3.48 ± 0.15	3.21 ± 0.12	4.25 ± 0.6	12.03 ± 0.43
	60	15.59 ± 1.43	3.48 ± 0.15[a]	3.26 ± 0.10	4.28 ± 0.06[a]	11.96 ± 0.29[a]

[a] $p < 0.05$ (placebo compared to homeopathic complex).
[b] For milk production, the baseline was at day 0, whilst milk constituents were assessed at day −2.

Table 3 Mean ± standard error for cortisol and glucose levels in plasma taken on days 0, 30 and 60 from cows treated daily with a homeopathic complex or placebo.

Group	Time point (days)	Cortisol (µg/dL)	Glucose (mg/dL)
Homeopathic complex	0	1.70 ± 0.47	50.42 ± 0.58
	30	0.98 ± 0.30	60.42 ± 1.21
	60	1.06 ± 0.17	67.42 ± 1.79
Placebo	0	0.93 ± 0.14	49.00 ± 1.29
	30	0.78 ± 0.17	59.42 ± 1.93
	60	1.83 ± 0.21[a]	67.58 ± 1.47

[a] $p < 0.05$ (placebo compared to homeopathic complex).

Table 4 Mean ± standard error for lactic acid and ammonia levels in plasma taken on days 0, 30 and 60 from cows treated daily with a homeopathic complex or placebo: no results are statistically significant ($p > 0.05$).

Group	Time point (days)	Lactic acid (mmol/L)	Ammonia (µmol/L)
Homeopathic complex	0	0.65 ± 0.14	92.35 ± 19.02
	30	0.72 ± 0.12	104.00 ± 16.02
	60	1.21 ± 0.21	129.67 ± 8.48
Placebo	0	0,63 ± 0.15	82.10 ± 18.04
	30	0.69 ± 0.11	86.25 ± 10.17
	60	1.66 ± 0.27	150.58 ± 23.09

more effective control of mastitis, generating a product with a greater added value by improving the quality of milk and allowing it to be designated as organic.[20] Several authors have verified the effectiveness of homeopathy in the control of bovine mastitis.[21–26] However, other studies obtained different results.[22,27] The study presented here provides preliminary supportive data for a novel homeopathic combination medicine in managing mastitis in cows that have established multi-resistance to antibiotics.

Among the most frequently isolated pathogens associated with bovine mastitis in Brazil, *S. aureus* is the most common.[28] Thus, two biotherapies (*S. aureus* and *Streptococcus* spp) were selected for inclusion in the novel homeopathic complex as the main etiological agents of mastitis.[29,30] In addition, the homeopathic medicine *Ignatia amara* was selected due to its affinity with stress conditions. The other medicines used in the homeopathic complex were selected based on their affinity for problems with lactation, including hard, swollen, painful and congestive teats (*Lac vaccinum defloratum*, *Phytolacca decandra*, *Urtica urens*); fistulous abscess and increased milk production (*Pulsatilla nigricans*); or inflammatory and infectious processes (*Calcarea carbonica*, *Asa foetida*, *Sulphur*).

Among the most-used methods for monitoring bovine mastitis is the SCC test. Somatic cells correspond to infiltrates in milk from the migration of defense cells of the blood to the interior of the mammary glands, in addition to desquamated epithelial cells, thus being an important tool in the diagnosis of the degree of infection of the mammary gland by the mastitic process.[31] Though there is no standard for determining SCC values in healthy, mastitis-free animals, the literature cites values between 100,000 and 200,000 cells/mL.[32] Brazilian legislation, through Normative Instruction Number 31, establishes that for milk to be considered fit for commercialization within the quality parameters, it must not exceed 400,000 cells/mL in the SCC test.[33] Thus, the SCC results obtained in the present study demonstrate that the milk from cows who received the homeopathic complex were close to the parameters considered normal for this test, and within the parameters of milk quality for commercialization in the country.

For SCC values lower than 450,000 cells/mL, dairy farmers receive a bonus of 1% for each reduction of 50,000 cells/mL; for values from 450,000 to 500,000 cells/mL there is no bonus; for values above 500,000 cells/mL they suffer a discount of 1% for each increase of 50,000 cells/mL; and above 750,000 cells/mL they suffer a total discount of 7% on the milk price.[34] In the present study, the animals in the placebo group presented SCC values of 726,000 cells/mL at 30

days and 1,520,000 cells/mL at 60 days of the experiment: values above those recommended by Normative Instruction Number 31.[33] The homeopathic complex group of animals maintained SCC at levels below 300,000 cells/mL. Low values of SCC help maintain the health of the mammary gland, and there is the possibility of a financial bonus to the producer, increasing profits in the commercialization of milk.

The improvement in the condition of the affected animals, and consequently in the quality of the milk, from the use of homeopathy was evidenced in the present study, with a lower SCC in the group treated with the homeopathic complex compared to the placebo group on day 60. The results are similar to those found in the literature,[29,30] noting that oral supplementation with homeopathic complexes promotes a significant change in the SCC of lactating cows, with a decrease in SCC in the treated group. This is also consistent with benefit seen for other homeopathic treatments. For example, in a study using a homeopathic complex in dairy cows, a decrease in sub-clinical mastitis was observed in animals treated with homeopathy when compared to the placebo group.[35] In dry cows treated with homeopathy, protection against mastitis was observed after subsequent calving.[36]

However, according to the literature,[37,38] the effectiveness of homeopathic protocols in reducing SCC in dairy cows has shown variation in the results, with some studies showing benefit and some studies not. These differences in outcome might be explained by some factors such as the lack of standardization in the preparation of homeopathic medicines, the form of administration, the dosages used, the pathogens present in each case, and the environmental conditions in which the study was conducted; all of which are important factors affecting the likelihood of deriving benefit from treatment.

It is also important to take into account that the healing process through homeopathy is believed to be substantially different from that which occurs in conventional therapies, with increases in SCC being common at the beginning of the use of homeopathy, due to the reaction of the organism that precedes the cure, which can have variable intensity and duration.[39] It is also important to pay attention to current legislation, which uses SCC as a determinant of milk quality.[40]

Regarding milk production, it is known that mastitis causes a decrease in this parameter[41] and even though there was an improvement in milk quality in the group treated with the homeopathic complex, there were no statistically significant differences between the homeopathic complex and placebo groups in the daily milk production. Corroborating our findings using a homeopathic complex in dairy cows, no significant difference was observed in milk production between the group treated with the homeopathic complex and placebo in another published study.[35]

During mastitis, total milk solids also undergo variations in their concentrations, mainly in relation to lactose and milk fat, which are usually reduced.[42] The decrease in milk solids levels occurs, for example, by lower synthesis of components caused by injury to mammary tissue [41] and also by leakage of components into the blood, through increased permeability of blood vessels during inflammation of the mammary gland.[42] In the present study, an increase in the levels of fat, lactose and total solids in the milk of the animals that received the homeopathic complex medicine suggests an active healing process within the tissue of the mammary gland, preventing leakage and loss of milk constituents. For the percentage of fat in milk, values above 3.40 are subsidized, with 6% for each percentage point; for proteins, values above 3.05 are subsidized, with 6% for each percentage point.[34] The homeopathic complex group in our study had higher fat values, and whilst this was not a large increase, according to Madalena 2000,[43] fat and protein are the components of milk with the highest economic value for dairy products, and their concentration is an indicator of the health of the herd and is associated with higher industrial yield of dairy products.

The effects on the quality of the milk verified in this study may be linked to the improvement of the immune response of the treated animals and by the reduction of the animals' stress, as evidenced by the plasma concentrations of cortisol. Cortisol is released under stress conditions and culminates in an impaired immune response,[44] making animals more predisposed to the development of mastitis, in addition to making the process of resolving the condition more difficult. Serum cortisol levels were lower in the homeopathic complex medicine group than the placebo group on day 60. Homeopathic medicines have been shown to change the behavior of animals by reducing stress.[45] In agreement with the results of the present work, other authors using a homeopathic complex in Nile tilapia fish verified a reduction in cortisol levels when compared to the placebo group.[46] Also, in sheep under conditions of feed restriction, there was observed a reduction in plasma cortisol levels and a higher level of antibodies in animals that received homeopathic complex in the diet.[47]

Serum lactic acid and ammonia are also considered to be indicators of stress. Lactic acid is a by-product of lactate, which is released by muscles under stress for the genesis of glucose.[48] Ammonia is derived from protein catabolism, which also results from the release of cortisol in stressors.[49] In the present study, however, no statistically significant differences between groups were found for these two parameters.

The animals used in our experiment evidenced multi-resistance to antibiotics: the animals of the group treated with homeopathy had a mean MAR index of 0.385 and the control group without treatment with homeopathy had a mean MAR index of 0.393. Values above 0.2 are considered high risk.[17] Corroborating these results, collecting and analyzing milk samples from dairy cows identified 19 isolates with a phenotypic profile of multidrug resistance.[50] Researching the resistance profile of 15 herds of dairy cows, where the use of antimicrobials for the treatment and prevention of bovine mastitis was reported in all the properties studied, 12 resistance patterns were observed, 6 of them being multi-resistance in nature. The most prevalent patterns were resistance to penicillin, ampicillin and tetracycline.[51] In another study, it was observed that five of the six *Escherichia coli* isolates collected from mastitic milk showed multi-resistance to the antimicrobials tetracycline,

neomycin and gentamicin.[52] In a survey carried out with 120 strains of *E. coli* isolated from bovine mastitis, it was shown that 20% of strains of this bacterium were resistant to two or more antimicrobials.[53] It was observed that penicillin, ampicillin, amoxicillin and neomycin are the antimicrobials to which the microorganisms that cause mastitis have greatest resistance.[54] The bacterial strains isolated from the milk of the animals in the present study showed resistance to eight different antimicrobials, indicating the clear need to seek new alternatives for the treatment of bovine mastitis.

Limitations of the study include a lack of randomization or blinding, as well as a small sample size. Randomization of the groups was not done as the animals used in the experiment are the university's dairy herd, whose management makes it difficult to randomize into experimental groups. Though a small number of animals per experimental group was used, the study still provides important preliminary data on the potential of a novel homeopathic combination product in an area of great clinical need.

Several classes of antimicrobials used in the treatment of diseases in animals are also used in human treatments, reinforcing the concern that the misuse of antimicrobials in animals can favor the development of antimicrobial resistance in humans.[55] The use of the homeopathic complex medicine in the present study was effective in the control of mastitis in multi-resistant antibiotic-resistant Holstein dairy cows. New studies should be carried out using double-blind randomized clinical trial design and with a larger number of animals per experimental group.

Conclusion

The use of a novel homeopathic complex medicine incorporated in the diet of dairy cows was found to have a promising impact on the SCC of milk, with a statistically significantly lower value than placebo at day 60. A significant increase in fat content, lactose and total milk solids at 60 days of treatment was also seen, with reduced serum cortisol levels in animals treated with homeopathy, making it possible to obtain superior quality milk, in addition to providing greater welfare of the production animals.

Highlights

- The study assessed the potential of a novel homeopathic complex medicine in managing bovine mastitis.
- After 60 days' treatment of dairy cows, SCC significantly increased in the placebo-treated group compared to the homeopathy-treated group.
- Compared to placebo, the group treated with homeopathy had reduced plasma cortisol, and higher percentages in milk of fat, lactose and total solids.
- The homeopathic medicine may have improved the immunological control of mastitis.

Supplementary material

Supplementary File 1. Ethics committee certificate.

Funding

This research was supported by Paranaense University. The authors thank the Minerphós Company for kind donation of the products.

Conflict of Interest

None declared.

References

1 Martins RP, Da Silva JAG, Nakazato L, et al. Prevalência e etiologia infecciosa da mastite bovina na microrregião de Cuiabá-MT. Cienc Anim Bras 2010;11:181–187
2 de Lima LNC, Torres LS, Da Silva LKB, et al. Avaliação microbiológica do leite cru e pasteurizado comercializado no município de Benevides-PA. Scientia Plena 2016;12:1–6
3 Fagundes H, Fernandes CAO. Infecções intramamárias causadas por *Staphylococcus aureus* e suas implicações em saúde pública. Cienc Rural 2004;34:1315–1320
4 Cades M, Zanini DS, de Souza HL, et al. Perfil de resistência antimicrobiana de mastite bovina em propriedade leiteira no município de Monte Negro/RO. Revista Brasileira de Ciências da Amazônia 2017;6:15–20
5 Costa Noel CC, Motta FS, Francisco NLSG, de Almeida NR, de Castro Soares L. Perfil de suscetibilidade antimicrobiana e produção de "slime" de isolados de Staphylococcus spp. provenientes de casos de mastite bovina na região sul-fluminense. Revista de Saúde 2016;7:22–26
6 Fukuya MH. Avaliação de multirresistência de *Staphylococcus aureus* de amostras de leite de vacas em assentamentos da região do Pontal do Paranapanema-SP. PhD dissertation. São Paulo, SP: Universidade de São Paulo; 2021
7 Chandrasekaran D, Venkatesan P, Tirumurugaan KG, et al. Pattern of antibiotic resistant mastitis in dairy cows. Vet World 2014; 7:389–394
8 Santé LF. Bactérias resistentes. O consumo de antibióticos está quase fora de controle. Brasil 247, May 15, 2018. Accessed June 10, 2022 at: https://fenafar.org.br/2022/05/17/bacterias-resistentes-o-consumo-de-antibioticos-esta-quase-fora-de-controle/
9 Silva SGM, Melo BA, Santos MT, et al. Resistance of *Staphylococcus aureus* and *Escherichia coli* to antibiotics. Research. Soc Dev 2022; 11:1–7
10 Cordioli E, Oldra A, Schmitt FA. Sistemas de produção de leite e qualidade do produto final na agricultura familiar. Cadernos de Agroecologia; 2009;4
11 Signoretti RD, Veríssimo CJ, de Souza FHM, et al. Aspectos produtivos e sanitários de vacas mestiças leiteiras tratadas com produtos homeopáticos. Arquivo Instituto de Biologia 2010;77:625–633
12 Costa NC, de Araújo RL, de Freitas GBL. Homeopatia: Um campo terapêutico fundamental no cuidado veterinário de animais de produção. Rev Salusvita 2009;3:73–89
13 Teixeira MZ. Fundamentação científica do princípio de cura homeopático na farmacologia moderna. Revista de homeopatia 2017;80:40–88
14 De Jesus RA, Coutinho CA. Uso de medicamentos homeopáticos para o tratamento da mastite bovina: Revisão. Pubvet 2017;12:1–10
15 Andrews RJ, Kitchen BJ, Kwee WS. Relationship between individual cow somatic cell count and the mastitis infection status of the udder. Aust J Dairy Technol 1983;38:71–74
16 Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 4 ed. Wayne: Approved Standard VET01–A4.CLSI; 2013
17 Blasco MD, Esteve C, Alcaide E. Multiresistant waterborne pathogens isolated from water reservoirs and cooling systems. J Appl Microbiol 2008;105:469–475

18 Farmacopéia Homeopática Brasileira 3rd ed. 2011. 300p. Accessed February 8, 2023 at: https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-homeopatica
19 Ayres M, Ayres Júnior M, Ayres DL, Santos AA. BioEstat: aplicações estatísticas nas áreas das ciências biomédicas. Belém: Ong Mamiraua; 2007
20 Peixoto ECTM, Pelanda AG, Radis AC, et al. Incidência de mastite bovina em animais homeopatizados. Rev Inst Laticínios Cândido Tostes 2009;64:66–71
21 Varshney JP, Naresh R. Comparative efficacy of homeopathic and allopathic systems of medicine in the management of clinical mastitis of Indian dairy cows. Homeopathy 2005;94:81–85
22 Nóbrega DB, Langoni H, Joaquim JGF, et al. Utilização de composto homeopático no tratamento da mastite bovina. Arq Inst Biol (Sao Paulo) 2009;76:523–537
23 Kiarazm M, Tajik P, Nava HG. Assessment of the effect of homoeopathic nosodes in subclinical bovine mastitis. Ann Biol Res 2011; 2:552–562
24 Defiltro RC, Glombowsky P, Girardini LK, et al. Addition of a homeopathic preventive product for mastitis in dairy cow feed: effects on etiologic agents, animal health, production, composition, and quality of milk. Research. Soc Dev 2020;9:1–30
25 Paim JB, Fraga DR, Libardoni F, et al. Avaliação de tratamento homeopático na prevalência da mastite bovina. Pubvet 2020; 14:1–5
26 Stoppe CV, Brassaloti CBP, Cândido CC, et al. The efficiency of homeopathy in the quality of bovine milk. Brazilian Journal of Development 2021;7:51305–51315
27 Mangiéri Júnior R, et al. Avaliação de tratamento homeopático na mastite bovina subclínica. Vet Zootec 2007;14:91–99
28 Silva MP. Identificação e caracterização de estirpes de *Staphylococcus aureus* e *Streptococcus agalactiae* associadas às mastites bovinas persistente e não persistente [Doctoral thesis]. Viçosa: Universidade Federal de Viçosa; 2015
29 Martins CR, Vieira EC, Gazim ZC, et al. Tratamento de mastite subclínica por meio de suplementação mineral homeopática da dieta de vacas leiteiras em lactação. Cultura Homeopática 2007; 19:16–19
30 Barzon CD, Medeiros F, Moraes RE, et al. Preliminary study of homeopathic treatment of subclinical mastitis evaluated through somatic cells count and California mastitis test. Int J High Dilution Res 2008;7:147–151
31 Campos R, Lacerda LA, Terra SR, González FHD. Parâmetros hematológicos e níveis de cortisol plasmático em vacas leiteiras de alta produção no Sul do Brasil. Braz J Vet Res Anim Sci 2008; 45:354–361
32 Harmon RJ. Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci 1994;77:2103–2112
33 Regulamento Técnico de Produção, Identidade e Qualidade do Leite tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel, alterada pela Instrução Normativa n° 7, de 3 de maio de 2016. Brasília, BR: Diário Oficial da União; July 2, 2018. Paper 2.
34 Bandeira A. Leite: Pagamento por qualidade a experiência do pool leite ABC. In: Seminário Estadual Sobre Qualidade do Leite, Castro, Paraná. 2004
35 Searcy R, Reyes O, Guajardo G. Control of subclinical bovine mastitis: Utilization of a homoeopathic combination. Br Homeopath J 1995;84:67–70
36 Klocke P, Ivemeyer S, Butler G, Maeschli A, Heil F. A randomized controlled trial to compare the use of homeopathy and internal Teat Sealers for the prevention of mastitis in organically farmed dairy cows during the dry period and 100 days post-calving. Homeopathy 2010;99:90–98
37 Keller D, Sundrum A. Comparative effectiveness of individualised homeopathy and antibiotics in the treatment of bovine clinical mastitis: randomised controlled trial. Vet Rec 2018;182:407
38 Zeise J, Fritz J. Use and efficacy of homeopathy in prevention and treatment of bovine mastitis. Open Agric J 2019;4:203–212
39 Mitidieiro AMA. Potencial do uso de homeopatia, bioterápicos e fitoterapia como opção na bovinocultura leiteira: avaliação dos aspectos sanitários e de produção [PhD dissertation]. Florianópolis, SC: Universidade Federal de Santa Catarina; 2004
40 de Almeida AC, Soares TMP, Silva DB, et al. Atividade de bioterápicos para o tratamento de mastite subclínica bovina. Rev Bras Agroecol 2011;6:134–141
41 Schultz LH. Somatic cells in milk-physiological aspects and relationship to amount and composition of milk. J Food Prot 1977; 40:125–131
42 Kitchen BJ. Review of the progress of dairy science: bovine mastitis: milk compositional changes and related diagnostic tests. J Dairy Res 1981;48:167–188
43 Madalena FE. Valores Econômicos para a Seleção de Gordura e Proteína do Leite. Rev Bras Zootec 2000;29:678–684
44 Marzotto M, Conforti A, Magnani P, Zanolin ME, Bellavite P. Effects of Ignatia amara in mouse behavioural models. Homeopathy 2012;101:57–67
45 Oppermann RV, Alchieri JC, de Castro GD. Efeitos do estresse sobre a imunidade e a doença periodontal. Rev Fac Odontol P Alegre 2002;43:52–59
46 Merlini LS, Vargas L, Piau R Jr, Ribeiro RP, Merlini NB. Effects of a homeopathic complex on the performance and cortisol levels in Nile tilapia (Oreochromis niloticus). Homeopathy 2014; 103:139–142
47 Chabel JC, Van Onselen JV, Morais MG, et al. Efeito de um complexo homeopático "Homeobase Convert H®" em ovinos sob condições de restrição alimentar. Braz J Vet Res Anim Sci 2009;46:412–423
48 Acco A. Mensuração dos niveis sericos de cortisol e de lactato desidrogenase como indicadores de estresse em cutia (Dasyprocta azarae) [PhD dissertation]. Curitiba, PR: Universidade Federal do Paraná; 1998
49 Alves AR, Figueiredo Júnior JP, Santana MHM, et al. Efeito do estresse sobre a qualidade de produtos de origem animal. Pubvet 2016;10:448–459
50 Ferreira LB, Israel LFS, Rabello RF, et al. Risk factors associated with the occurrence of multiresistant Staphylococcus spp. isolated from bovine subclinical mastitis in northern Brazil. Semin Cienc Agrar 2022;43:901–910
51 Dias JA, Menezes CA, Brito MAVP, et al. Antimicrobial resistance profile of Staphylococcus spp. isolates in cattle herds from Western Amazon. Semin Cienc Agrar 2022;43:1355–1364
52 Moreira MAS, Ferreira AB, Trindade TFSL, et al. Resistência a antimicrobianos dependente do sistema de efluxo multidrogas em *Escherichia coli* isoladas de leite mastítico. Arq Bras Med Vet Zootec 2008;60:1307–1314
53 Ribeiro MG, Costa EO, Leite DS, et al. Fatores de virulência em linhagens de Escherichia coli isoladas de mastite bovina. Arq Bras Med Vet Zootec 2006;58:724–731
54 Acosta AC, Silva LBG, Medeiros ES, et al. Mastites em ruminantes no Brasil. Pesqui Vet Bras 2016;36:563–573
55 Freitas GD, Lima CP, Coelho DFS, et al. Uso de diferentes métodos no controle do desenvolvimento do *Staphylococcus aureus*: uma revisão da literatura. Research. Soc Dev 2021;10:e40310212546

