Original article

Evaluation of homeopathic protocols for the treatment of subclinical mastitis in lactating cows

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Abstract

Introduction: We analyzed the effects of a homeopathic therapies to control subclinical mastitis in two dairy herds. **Materials and Methods:** Two experimental herds were used, one in the municipality of São Carlos, SP (Herd A) and the other in Bagé, RS (Herd B), with 46 and 37 lactating cows, respectively. Milk yield, somatic cell count (SCC), and levels of fat, protein, lactose and total solids were evaluated. Milk samples were collected for microbiological confirmation of mastitis. The lactating cows were divided into four groups formed on each of the two farms: cows with mastitis, treated and untreated, and healthy cows, also with and without homeopathic treatment. The occurrence of mastitis was compared between groups using the chi-square test. Means of continuous variables were compared by means of the Tukey-Kramer test. The herd effect on SCC, milk yield, and other compositional aspects was determined in treated and untreated animals, using multiple correspondence analysis. **Results:** No difference was found in the occurrence of mastitis between treated and untreated animals in Herd B, but in Herd A, mastitic cows were more commonly cured in the untreated group ($P<0.05$). No differences were detected in milk production and composition between treated and untreated cows with mastitis. Differences in SCC were observed only between mastitic and healthy animals. **Conclusions:** No correspondence was found between the treated and untreated groups. Overall, our findings suggest that homeopathic treatment did not improve milk quality and production of experimental animals.

Keywords: homeopathic treatment; lactation; somatic cell count*; staphylococci*

Introduction

Milk production on farms with organic management has aroused interest in the dairy sector, but disease control in this type of management is crucial, due to limited use of antimicrobials. Mastitis is the most common disease in dairy herds, causing significant economic losses due to declining milk production and quality [1].

One of the goals of both organic and traditional farms that use antimicrobial products is the longevity and improvement of sanitary aspects of the udder, which makes the prevention of new infections particularly important [2]. However, the somatic cell count (SCC), an indicator of mammary gland health, may be higher at organic farms that do not use conventional antimicrobials. Also, mammary gland conditions worsen throughout the productive life of animals in organic herds because of chronic infections resulting from the limited use of antibiotics in multiparous cows [3]. This may prove to be problematic since animals with numerous parities are not always discarded in Brazil.

Additionally, the farms under organic management present more difficulties for the control of contagious microorganisms involved in mastitis than in conventional herds, due to the non-use of dry cow therapy. On these farms, strategies to reduce intramammary infection during the dry period are scarce [2, 3]. Therefore, this study focused on evaluating the effects of homeopathic therapies employed to control subclinical mastitis in two dairy herds subjected to this type of treatment.

Materials and Methods

Characteristics of the farms and herds

The herds of this study came from two farms, one located in São Carlos, state of São Paulo, Brazil (Herd A) and the other in Bagé, state of Rio Grande do Sul, Brazil (Herd B). Herd A had 46 lactating cows before treatments (D0), Holstein and Holstein x Jersey, who's grazed on *Panicum maximum* cv. Tanzania, concentrate feed and cottonseed. Mechanical milking was performed twice a day in a closed-circuit milking system. Average daily milk yield was 28 liters. The cows to be treated and those not treated with homeopathy had microbial isolations at the beginning of the experiment. Among the treated animals, *Corynebacterium* spp. was the most frequent microorganism, followed by coagulase-negative staphylococci (CNS) and coagulase-positive staphylococci (CPS). Untreated cows showed isolation of *Corynebacterium* spp., *Streptococcus* spp. and *S. aureus*. The mean SCC on D0 was 74,000 and 67,000 cells/mL of milk in the treated and untreated cows, respectively.

Herd B had 37 lactating Dutch and Jersey dairy cows. Mechanical milking was performed twice a day, at also in a closed-circuit milking system. The cows were fed on native pasture overseeded with ryegrass, oat pasture in the fall-winter seasons, concentrated feed, as well as corn and hay silage (ryegrass and/or birdsfoot deervetch [*Lotus corniculatus*]) supplied during forage shortages. The average daily milk yield of the Dutch dairy cows was 17 liters while that of the Jersey cows was 11 liters. The mean SCC on D0 was 94,000 and 99,700 cells/mL in treated and untreated animals, respectively. The isolation of coagulase-negative staphylococci (CNS) was verified in one treated animal, while in the untreated animals, there was an absence of microorganisms.

The two herds underwent the same procedures before, during, and after milking. Herds were chosen because they belong to farms of scientific research institutions, where the conditions of the experiments could be more controlled.

Collection of samples for bacteriological diagnosis of mastitis

Milk from the four quarters of the udder was collected and pooled for microbiological analysis, as recommended by the National Mastitis Council [4]. The milk samples were collected in duplicate and sent to the laboratories located on the same farms where the herds were raised. Animals in the first 10 days postpartum were not selected for participation in the study. After this period, samples were collected monthly to monitor the etiology of mastitis, concurrently with homeopathic treatments.

Physicochemical tests, somatic cell count, and milk yield

Milk yield, SCC, total solids, fat, protein, and lactose were evaluated monthly. In herd A, milk yield per cow was measured after completely milking each animal, using graduation marks on the milk lines of the milking machine. In herd B, total milk yield was recorded in the morning, after completely milking each animal, based on electronic liquid volume measurements taken by the mechanical milking machine. Samples were sent to laboratories of the Brazilian Network of Milk Quality Control Laboratories for the analysis of SCC, total solids, fat, protein, and lactose.

Distribution of the animals in the groups

Four groups were formed on each of the two farms, these groups comprised cows with mastitis, treated and untreated, and healthy cows, also with and without homeopathic treatment. Half the

animals in Herd A underwent homeopathic treatment, while the other half were treated with a placebo. In Herd B, the treated group comprised 19 animals, and the untreated group contained 18 cows. Milk production values were considered to ensure that both groups were balanced before the treatments, as to the prevalence of the disease [5].

Homeopathic treatment

The choice of active ingredients for the homeopathic treatment was based on knowledge of the infectious etiology of mastitis and pure medical materials that resemble symptoms of mastitis [6]. A specialist in homeopathy explained how to prepare and use the homeopathic formulation for treating cows, using the active ingredients *Belladonna* (12CH), *Hepar sulphur* (12CH), *Silicea* (12CH), *Phosphorus* (12CH) and *Phytolacca decandra* (12CH) of the homeopathic compound was added to the feed of the lactating cows (Herd A), as directed, while the untreated animals received a placebo of sugar added to their feed. The homeopathic formulation (5 mL) was diluted in crystal sugar (500 g). This mixture was combined with 30 kg of salt. This concoction was then mixed into the concentrate and fed to the animals, 2 kg/animal/day for 12 months. In Herd B, the homeopathic remedy was administered in the form of two jets of spray (approximately 120 µL/jet), applied directly onto the vulva of each cow twice a day, while the animals in the untreated group received a placebo. The treatment was applied for six months.

Monitoring the evolution of mastitis in treated and untreated cows

The animals were classified as infected when *Staphylococcus aureus* or *Streptococcus* spp. were isolated after treatment, and coagulase-negative staphylococci (CNS) and/or *Corynebacterium* spp. were detected in milk with SCC starting at from ≥ 200,000 cells/mL. On the other hand, cows were considered healthy when no microorganisms were detected, or in cases where CNS and *Corynebacterium* spp. were isolated jointly with SCC lower than 200,000 cells/mL [7].

Analysis of data

The occurrence of healthy and diseased animals in the treated and untreated groups was analyzed by the chi-square test to ascertain independence between the groups. Data on SCC, milk yield, protein, lactose, fat, and total solids were analyzed using descriptive statistics in the herd A [8,9]. These variables were subjected to analysis of variance by the MIXED procedure in the Statistical Analysis System [10], following a completely randomized design. The model considered the effects of treatment, month, and Treatment-Month interaction, in a split-plot design over time, using the months as a repeated measure. The LSMEANS function was adopted for the multiple comparisons between means, using Tukey's test with a 5% level of significance. The variable SCC was log-transformed. Multiple correspondence analysis (MCA) was used as an exploratory statistical approach to examine associations or similarities between the variables of milk composition, yield, and SCC in treated and untreated cows of both herds. The basic concept of MCA stems from the Chi-square statistic and is expressed by the formula:

$$\chi^2 = \sum_{ij} \frac{\left(n_{ij} - e_{ij}\right)^2}{e_{ij}} = \sum_{ij} \frac{\left(n_{ij} - n_{i.} n_{.j}/n\right)^2}{n_{i.} n_{.j}/n}$$

with (I-1)(J-1) degrees of freedom, where: *eij* = *ni.n.j* /*n* is the "expected" value in the cell (*i,j*) of the matrix, based on the marginal rows and columns *ni.* and *n.j*, respectively, of a contingency table. A 5% level of significance was adopted [11].

Results

Table 1 describes the occurrence of subclinical mastitis in the herds.

Table 1 Occurrence of mastitis in mammary quarters from cows treated and not treated with the homeopathic formulation, during 12 and 6 months of sample collection in herds A and B, respectively.

	Herd A[1]			Herd B[2]		
	Healthy	Mast[3]	Total	Healthy	Mast[3]	Total
Untreated	205	75	280	103	4	107
	73.21 %	26.79 %	100%	96.26%	3.74%	100%
Treated	190	100	290	104	7	111
	65.52%	34.48%	100%	93.69%	6.31%	100%
Total	395	175	570	207	11	218
	69.3%	30.7%	100%	94.95%	5.05%	100%

[1] P=0.046; [2] P=0.3865; [3] Mastitic cows

In Herd B, treated and untreated cows showed no differences in the occurrence of mastitis. However, in Herd A, the proportion of cows with mastitis was higher among treated animals (P=0.046).

Milk production of non-mastitic cows treated with homeopathy was higher than that of untreated mastitic cows (P <0.05) (Table 2), but no differences were found between healthy cows with and without treatment and infected animals with and without treatment (P>0.05).

Table 2 Milk yield, somatic cell count, and milk composition of cows with and without mastitis treated and not treated with homeopathic medicine.

	Herd A				Herd B			
	Untreated		Treated		Untreated		Treated	
	Healthy[1]	Mast[2]	Healthy[3]	Mast[4]	Healthy[1]	Mast[2]	Healthy[3]	Mast[4]
Yield (L)	21.55^{ab*}	19.28^{a}	24.04^{b}	22.48^{ab}	18.4	20.31	19.61	19.15
SCC (cells x 10^3)	264^{a}	650^{b}	248^{a}	799^{b}	136	67	143	191
Fat (%)	2.90^{a}	3.18^{a}	3.04^{a}	3.14^{a}	3.13	3.29	3.11	3.86
Protein (%)	3.34^{a}	3.43^{a}	3.27^{a}	3.38^{a}	3.14	3.02	3.13	3.16
Lactose (%)	4.55^{a}	4.38^{a}	4.60^{a}	4.48^{a}	4.51	4.55	4.37	4.46
Total solids (%)	11.75^{a}	11.97^{a}	11.86^{a}	11.95^{a}	11.63	11.59	11.46	12.38

[1] Untreated healthy cows; [2] Untreated mastitic cows; [3] Treated healthy cows; [4] Treated mastitic cows

[*] Same letters between columns (P> 0.05).

The SCC was higher in mastitic cows, regardless of whether they were treated. Considering the variable of milk yield, no differences were found between treated and untreated healthy animals and treated and untreated mastitic cows. Nor were differences detected in the variables of fat, protein,

lactose and total solids. The low occurrence of the disease in Herd B prevented an analysis of the variables under study, given the insufficient number of repetitions for data investigation.

Reinfection rates, new cases and cure rates are relevant indicators of the efficiency of treatment and management of animals. On D0 the rates of subclinical mastitis were 22% and 26% in the control and treated groups, respectively (Table 3). During the evaluated periods, the percentages of subclinical mastitis were higher in treated cows, except at the beginning of month 7, month 8 and month 11.

Table 3 Epidemiological indicators (%) of mastitis in herd A.

	Subclinical mastitis[1] (%)		**Reinfection[2] (%)**		**New cases[3] (%)**		**Cure rates[4] (%)**	
	C[5]	**T[6]**	**C**	**T**	**C**	**T**	**C**	**T**
D0	22	26	-	-	-	-	-	-
Month 1	16	40	-	-	11	29	60	17
Month 2	8	36	0	0	5	7	75	20
Month 3	22	26	67	100	24	3	100	42
Month 4	19	33	0	33	12	19	50	20
Month 5	40	42	100	100	25	23	100	20
Month 6	0	29	0	0	18	10	13	33
Month 7	39	22	0	33	13	13	14	50
Month 7*	31	39	0	67	19	44	56	25
Month 8	32	27	40	100	26	19	43	50
Month 9	27	30	67	40	25	24	67	40
Month 10	32	39	50	0	20	19	33	14
Month 11	39	28	100	100	33	8	40	56

[1] Positive microbiological culture; [2] Compared to the previous two months;[3] Compared to the previous month (maybe in conjunction with reinfections). It does not exclude cases of mastitis in the interval between collections;[4] Compared to the immediately preceding month; [5] Control group/not treated; [6] Treated group; * Two collections in the same month, one at the beginning and one at the end.

Treated and untreated cows had cases of clinical mastitis in Heard A. In all, there were 19 cases in the untreated group and 11 cases among the animals treated with homeopathy. Despite the higher occurrence of clinical cases among untreated cows, the number of cows with clinical mastitis in each group was similar, i.e., eight untreated and nine treated cows.

Figure 1 illustrates the associations between milk yield, SCC, and other variables related to milk composition, according to herd and mastitis treatment.

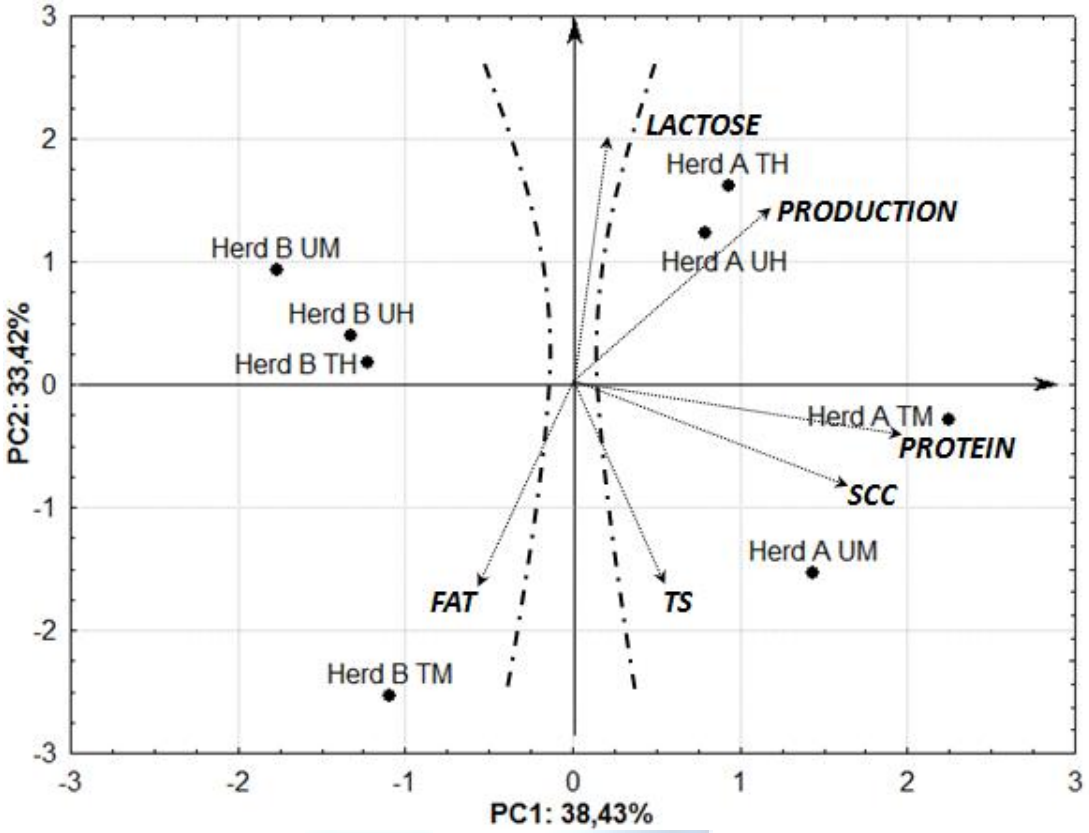

Fig. 1 Categories of qualitative variables and their associations, according to herd and treatment or nontreatment of mastitis (UM: Untreated mastitic cows; UH: Untreated healthy cows; TM: Treated mastitic cows; TH: Treated healthy cows).

The two-dimensional map explains 71.9% of the total variance. It was found that just as cluster analysis identified the difference between the two herds, the principal component analysis separated Herd A and Herd B on opposite sides. In Herd A, higher lactose content and milk production were associated with the absence of mastitis (Herd A TH and Herd A UH), regardless of whether the animal belonged to the group of untreated cows or the group treated with homeopathy. Conversely, higher SCC and protein levels in Herd A involved both treated and untreated mastitic cows.

Discussion

In the present study, a placebo was used for the control group instead of allopathic treatment, since the conventional treatment of subclinical mastitis during lactation has low efficiency, despite the damage caused by this type of disease, in addition to the need to discard the milk from treated cows. In contrast, [12] compared homeopathy and conventional treatment and reported results of similar cure rates, but with no difference compared to placebo. We opted for the coexistence between cows not treated and treated at milking time because they could not be separated in the herds, due to the management conditions. This coexistence was the possible way to allow comparison between cows treated and untreated with homeopathy.

The choice of the homeopathic formulation in this study was based on previous knowledge about the infectious etiology of mastitis, caused mainly by contagious microorganisms involved in subclinical cases of the disease. In another study such as the one conducted by [13] used a mixture of 4 nosodes

intravaginally. According to [14], this type of homeopathic treatment is less effective when compared to the one used in the present study.

The practice of classifying a condition of mastitis together with the animal's SCC is considered essential to identify subclinical mastitis. Moreover, an infectious condition of subclinical mastitis is usually tied in with an SCC of more than 200,000 cells/mL of milk [3]. Thus, in the present study, we decided to consider spontaneous cures as the absence of isolation of microorganisms in conjunction with the decrease in the SCC to less than 200,000 cells/mL of milk in untreated cows, which reflects this classification more confidently. Some cows close to drying up, with low milk yield, were not removed before the data were analyzed to avoid bias in favor of either group. These animals were found to be mainly in the untreated group, which may have influenced the SCC. Other authors [14] indicate that the cure rates of animals undergoing homeopathy treatment were 28%, a value indicative of the inefficiency of the homeopathic treatment. According to [13], reinfections among treated cows were more frequent than in untreated animals.

An upward trend in milk SCC was reported [15] when a homeopathic combination was given to cows with good mammary gland health, which, according to experts in the field of homeopathy, could increase the responsiveness to intramammary infections in treated animals. Thus, the SCC of milk may remain high even in healthy animals. However, in Herd A, where there was a difference in the proportion of treated and untreated mastitic and healthy animals, there were healthy cows with SCC above 200,000 cells/mL and without microorganisms in milk, not only among animals treated with homeopathy but also among untreated cows. Moreover, this possible increase in SCC reported by other authors in animals treated with homeopathy may be detrimental to milk production, since prolonged leukocyte diapedesis may damage the mammary parenchyma [16].

The low occurrence of the disease in Herd B prevented an analysis of the variables under study, given the insufficient number of repetitions for data investigation, which was a limiting factor in this study. Concomitantly, according to the results described in Table 1, a comparison of the proportion of mastitic and healthy animals and treated and untreated groups showed no difference in Herd B.

The two-dimensional map (Figure 1) explains that mastitis negatively influences a cow's milk yield and lowers lactose levels in diseased cows, a physiological response of the mammary gland triggered by an inflammatory response. This inflammatory response is also responsible for higher cell counts in diseased animals and an increase in serum protein levels [17, 18, 19]

Conclusions

Homeopathic treatment was not effective against subclinical mastitis or improved the milk quality of the treated cows under the experimental conditions of this study. Milk production did not increase in cows treated for mastitis.

Declarations

Ethics approval and consent to participate

The experiment was conducted according to animal welfare practices and approved by the Ethics Committee on the Use of Animals of Embrapa Southeastern Livestock (Protocol CEUA 02/2014).

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions
LFZ conceived and designed research, conducted experiments, performed the laboratory analysis, and wrote the manuscript. APM, EBG, RWSMS, and RD conducted experiments and performed the laboratory analysis; FDC wrote the manuscript and reviewed the results; TCA conducted experiments and conceived and designed research; WBJ analyzed data. All authors read and approved the manuscript.

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Conflict of Interest: Authors declares there is no conflict of interest.

Financial Suport: We had full access to all the data in this study and we take complete responsibility for the integrity of the data and the accuracy of the data analysis.