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Negatively controlled, randomized clinical trial to evaluate a culture-based selective antimicrobial treatment of non-severe clinical mastitis on behavior of dairy cows

[*Ensaio clínico com controle negativo para avaliar o efeito do tratamento seletivo de mastite clínica não severa baseado em cultura microbiana sobre o comportamento de vacas leiteiras*]

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ABSTRACT

The aim of the present negatively controlled, randomized clinical trial was to assess dry matter intake (DMI) and behavior in cows with mild and moderate naturally occurring CM caused by gram-negative or culture-negative pathogens. Enrolled cases were assigned to either the negative control or to the treatment group, which received one infusion of intramammary ciprofloxacin for three consecutive days. Behavior was assessed through neck collars which monitors rumination, activity, and rest times. Electronic feeders were used to record feeding behavior and DMI. Also, average daily milk yield, milk composition, somatic cells count (SCC), and white blood cells count (WBC) were evaluated. Of a total of 67 cases of CM, 23 met the criteria to enter the experiment and were enrolled to the negative control (n=13) and to the treated group (n=10). There was no difference in behavior between groups throughout the studied period. Also, no difference was found in DMI, feeding behavior and in the other parameters between negative control and treated cases. Therefore, the selective treatment did not have negative impacts on behavior and DMI, as well as in the other parameters. This therapeutic strategy can be beneficial since reduces costs and the use of antimicrobials in dairy farms.

Keywords: antimicrobial, behavior, dairy, feed intake, mastitis

RESUMO

O objetivo do presente ensaio clínico foi avaliar o consumo de matéria seca (CMS) e o comportamento de vacas diagnosticadas com mastite clínica (MC) leve ou moderada, provocada por patógenos Gram negativos ou com cultura negativa. Os casos foram designados para o grupo controle negativo ou grupo tratamento, o qual recebeu uma infusão intramamária de ciprofloxacino por três dias consecutivos. O comportamento foi avaliado por meio de coleiras que monitoram os tempos de ruminação, de atividade e de ócio. Cochos eletrônicos foram utilizados para registrar o comportamento alimentar e o CMS. Além disso, a produção média diária de leite, a composição do leite, a contagem de células somáticas e a contagem de leucócitos também foram avaliadas. A partir de 67 casos de MC, 23 atenderam aos critérios de inclusão e foram designados para o grupo controle negativo (n=13) e para o grupo tratamento (n=10). Não foram encontradas diferenças no comportamento entre os grupos, durante o período experimental. Ademais, não houve diferença para o CMS, o comportamento alimentar e os demais parâmetros, entre o controle negativo e os casos tratados. Assim, o tratamento seletivo não trouxe impactos negativos sobre os parâmetros estudados. Essa estratégia terapêutica pode ser benéfica por reduzir custos e o uso de antimicrobianos, nas propriedades leiteiras.

Palavras-chave: antimicrobianos, comportamento, consumo de matéria seca, mastite, pecuária leiteira

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INTRODUCTION

Mastitis is the most prevalent disease in dairy herds worldwide (Pinzón-Sánchez *et al*., 2011; Ruegg, 2017.). The bulk of antimicrobials used in dairy herds is directly associated with mastitis, either for therapeutic or prophylactic measures (Pol and Ruegg, 2007; Campos *et al*., 2021). It is imperative to develop alternatives to antibiotics and new therapeutic strategies, such as culture-based selective treatment protocols to reduce the risks of antimicrobial resistance (Lago *et al*., 2011a; Sharun *et al*., 2021; Ajose *et al*., 2022; Morales-Ubaldo *et al*., 2023).

Furthermore, mastitis has a strong negative economic impact on dairy herds (Gonçalves *et al*., 2023; Morales-Ubaldo *et al*., 2023). Direct costs associated with this disease are medicines, milk disposal, and labor, while indirect costs are reduction in milk production, decreased milk quality, reduced reproduction performance, and early culling of cows (Rollin *et al*., 2015). Guzmán-Luna *et al*. (2022) observed an annual loss of row milk due to mastitis reaching 2.15% in the main dairy production regions of Europe. Previous studies found an impact of US$70.30 per cow per year (Romero *et al*., 2018) and a cost of US$444.00 per episode occurring during the first 30 days of lactation (Rollin *et al*., 2015). Guimarães *et al*. (2017) conducted a study in a Brazilian dairy herd with 169 lactating cows and found a financial loss of approximately US$60,000.00 per year in which treatment and discarded milk costs contributed to almost 20% of the total cost.

Clinical mastitis (CM) is often classified based on the severity of the inflammatory response. In mild cases, only abnormal milk is observed, while in moderate cases, these signs are accompanied by udder inflammation signs, such as redness, heat, swelling, and pain (Pinzón-Sánchez *et al*., 2011). Lastly, cows with severe cases of mastitis will experience systemic signs, including fever, anorexia, and dullness (Cheng and Han, 2020). Another factor that adds to the complexity of this disease is the vast number of microbials capable of infecting the mammary gland. The use of on-farm culture has enabled the correct and fast identification of pathogens causing mastitis in dairy herds, which in turn allowed the adoption of strategic therapies (Lago *et al*., 2011a).

Recent studies showed no benefit in treating mild and moderated cases of mastitis with intramammary (IMM) antimicrobial when the microbiological culture yielded gram-negative bacteria or no growth (Pyörälä *et al*., 1994; Oliveira *et al*., 2015; Ruegg, 2021). Clinical and bacteriological cure rates, post-treatment SCC, milk yield, recurrence, and culling were all investigated under different treatment protocols and there were no improvements when comparing clinical cases treated using blanket therapy and selective treatment strategies (Lago *et al*., 2011a, 2011b; Fuenzalida and Ruegg, 2019a).

Moreover, mastitis is also known to impact behavior and feed intake of affected animals (Fogsgaard *et al*., 2012). Studies conducted with mastitic and healthy animals observed that cows with CM spent less time lying down, had decreased level of activity, and showed restlessness signs during milking (Medrano-Galarza *et al*., 2012; Fogsgaard *et al*., 2015). Sepúlveda-Varas *et al*. (2016), described decreased feed intake and changes in the feeding behavior of cows affected with CM.

The aim of the present negatively controlled clinical trial was to evaluate the effect of a culture-based selective IMM treatment with a negative control on dry matter intake (DMI), feeding time, number of feeder visits, and other feeding behaviors, as well as rumination, activity, and rest times of gram-negative and culture-negative mild and moderate cases of CM.

MATERIALS AND METHODS

A negatively controlled, randomized clinical trial was conducted between May and October 2020 in a commercial dairy herd located in the south of Brazil. The herd contained approximately 400 lactating dairy cows. All the animals used in the experiment were multiparous Holstein cows, milked twice a day, housed in a compost bedded-pack barn with free access to water, and were fed the same diet as a total mixed ration (TMR), according to the NRC, 2001. All experimental procedures were approved by the Animals Ethics and Experimentation Committee of the Federal University of Pelotas (no. 14122-2020).

Detection of new CM cases was performed in the milking parlor by trained farm personnel and researchers during foremilk. The severity of the clinical cases was determined by researchers based on clinical examination and milk observation. Cases with abnormal milk, such as color and consistency alteration, as well as the presence of clots and altered secretions were defined as mild. When abnormal milk was accompanied by udder inflammation signs, such as swollen, hot, and painful quarters, the case was defined as moderate. Severe cases were detected when cows exhibited systemic signs, including pyrexia, dehydration, anorexia, and dullness.

Mild and moderate cases of CM caused by gram-negative bacteria or with no growth in the culture media were eligible for enrollment in the experiment. Cases classified as severe were not enrolled and were immediately treated with the protocol designated by the farm. Clinical cases initially defined as mild or moderate, which evolved to severe (started exhibiting systemic clinical signs), were removed from the study and received treatment according to the farm protocol. Cases with the presence of clinical signs of any type or which had received antimicrobial treatment 15 days prior to the identification, as well as cases with concurrent existence of another disease were not eligible for the study. Randomization was achieved using the function SAMPLE of R Studio version 4.2.0 (RStudio Team**,** RStudio: Integrated Development for R. RStudio, PBC, Boston, 2020) to generate a predefined order.

Immediately after enrollment, milk samples were aseptically collected from affected quarters by researchers to perform the on-farm diagnosis. The on-farm pathogen identification was performed using a triplate chromogenic culture media (SmartColor 2, Onfarm) which capable to detect the main pathogens that cause mastitis: *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberi*s, *Enterococcus spp*., *Lactococcus spp*., *Staphylococcus aureus*, non-*aureus Staphylococci* (SNA), other gram-positive, *Escherichia coli*, *Klebsiella* spp. *Serratia* spp., *Pseudomonas* spp., *Prototheca* spp., yeast, and other gram-negative. Granja *et al*. (2021) conducted a study to investigate the diagnostic performance of this on-farm triplate method and found an adequate accuracy for identification of the main bovine mastitis causing microorganisms. First, a sterile cotton swab was dipped into the sterile plastic tube where the milk sample was stored. Subsequently, the milk aliquot was inoculated into the tri-plate containing the selective chromogenic media, using a different swab for each section of the plate. Afterwards, the culture plate was incubated at 37°C for a period of 24 hours and then, the results interpretation was performed according to the manufacture guidelines. There were five possible results: (1) gram-positive bacteria; gram-negative bacteria; (3) both gram-positive and gram-negative bacteria; (4) no growth (when the culture yielded no bacteria); (5) contaminated (three or more pathogens).

After enrollment, cases that met the criteria were randomly assigned to either treatment or negative control. Quarters assigned to treatment received a daily IMM infusion of ciprofloxacin (Ciprolac, Ouro Fino Saúde Animal, Brazil) for three consecutive days, whereas quarters assigned to negative control did not receive any type of treatment.

Data was collected from each animal for the period of nine days following the CM case detection (d0). Animal behavior was assessed through neck collars equipped with accelerometers (C-TECH Chip-Inside, CowMed, Brazil) which monitors rumination, activity, and rest times, 24 hours per day. Rumination was detected by lateralized head movements, typically observed during the process of regurgitating, rechewing and re-swallowing the partially digested feed. Activity was detected as any type of movement, except the one considered rumination. Lastly, the rest time was obtained subtracting rumination and activity times, where the animal will not necessarily be lying down. Feeding behavior was assessed through electronic feeders (Eletronic Feeder AF1000, Intergado, Brazil) which measures the feed intake, feeding time, number of feeder visits, and number of feeder visits with consumption. Feed samples were collected daily to obtain the dry matter content and calculate the dry matter intake (DMI).

Daily milk production records from individual cows were retrieved from the farm record system (DelPro, DeLaval, Sweden). Milk samples were collected at d0 and d8 from all quarters of enrolled animals and were sent (conserved with bronopol) to a commercial laboratory

(Unianálises, Laboratório de Análises e Prestação de Serviços – UNIVATES, Lajeado, RS, Brasil) to perform milk SCC and composition (lactose, fat, and protein) analyses. Blood samples were collected at d0, d5 and d8, in relation to the CM cases identification, through coccygeal vein puncture using a vacutainer system in tubes containing EDTA. Subsequently, these samples were stored under refrigeration and then transported to a laboratory to obtain white blood cells count (WBC). The analysis was performed using an automated counter (BC-2800 Vet, Mindray, China).

All statistical analyses were conducted using SAS software (SAS Studio 3.5, SAS Institute, USA, 2016) and values of $P < 0.05$ were considered significant. The SCC was log transformed to follow normal distribution. Categorical explanatory variables were severity (mild or moderate), parity (2^{nd} or $\geq 3^{rd}$), pregnancy status (yes or no), DIM (0-100 and >100), and on-farm culture result (gram-negative or no growth). PROC FREQ was used to perform Chi-Square test and Fisher's Exact test between negative control and treated for categorical variables at enrollment. Continuous variables were rumination, activity, and rest times, DMI, feeding time, number of visits to the feeder, number of visits to the feeder with consumption, milk yield, SCC, milk composition (protein, fat, and lactose), and WBC count. PROC MIXED MODELS was used to perform mixed ANOVA analysis to compare the continuous variables between negative control and treated.

RESULTS

During the experimental period, 67 cases of CM were identified, of which 23 met the criteria to entry the study. In total, 44 cases of CM were excluded from the study, 17 cases yielded gram-positive bacteria in the culture media, 5 cases were classified as severe, and 22 other cases were excluded from the experiment since data of behavior and feed intake was not available for the entire period of nine days. From cases which met the criteria, 10 were assigned to treatment and 13 were assigned to negative control (Fig. 1).

The distribution of severity, parity, pregnancy status, DIM, and on-farm culture result is shown in Table 1. No difference was found between negative control and treated for all categorical variables. Mean and median DIM were 167 and 161, respectively. Among gram-negative bacteria yielded in the on-farm culture, there was *E. coli*, *Klebsiella* spp., and other gram-negative.

Rumination, activity, and rest times were not different between treated group and negative control throughout the observational period of nine days after the detection of clinical signs (Table 2).

There was no difference of DMI between cows in the negative control (23.02±1.17Kg) and those treated with IMM antimicrobials (22.55±1.29 kg), however it was observed an increase in DMI one day after the onset of clinical signs in both negative control and treated cases of CM ($P<0.001$, Fig. 2).

Negative control and treated cases did not differ in relation to the feeding behavior (Tab. 3). Nevertheless, it was found the same pattern observed in DMI, since there was an increase for both experimental groups in the first 24 hours upon case detection in feeding time ($P < 0.001$)., number of visits to the feeder ($P < 0.001$)., and number of visits to the feeder with consumption ($P = 0.002$).

There was no difference in the average daily milk yield (43.15±2.36 and 42.06±2.70kg in the negative control and treatment, respectively; $P = 0.87$, Fig. 3). Also, there was no difference in the milk composition. Average lactose, fat, and protein concentration were 4.1%±0.1 and 3.8% ± 0.1 ($P = 0.26$), 2.0% ± 0.3 and 2.4% ± 0.4 ($P = 0.46$), and 3.4±0.8 and 3.3 ± 0.9 ($P = 0.52$) for cows in the negative control and treated cases, respectively. The average lactose concentration increased from d0 to d8 in the negative control ($P = 0.009$). No difference was observed in SCC (6.31 ± 0.1 and 3.8 ± 0.1 $\log_{10}$SCC in the negative control and treated cases, respectively), however there was significant reduction in the negative control ($P < 0.05$) and a trend towards reduction in treated cases ($P = 0.08$) between d8 and d0. WBC did not different between experimental groups (13,764 ± 1,635 and 10,351 ± 1816 cells/µL in the negative control and treated cases, respectively), yet there was an increase in the negative control, between d5 and d8 ($P = 0.007$) and in the treated group, between d0 and d8 ($P = 0.05$).

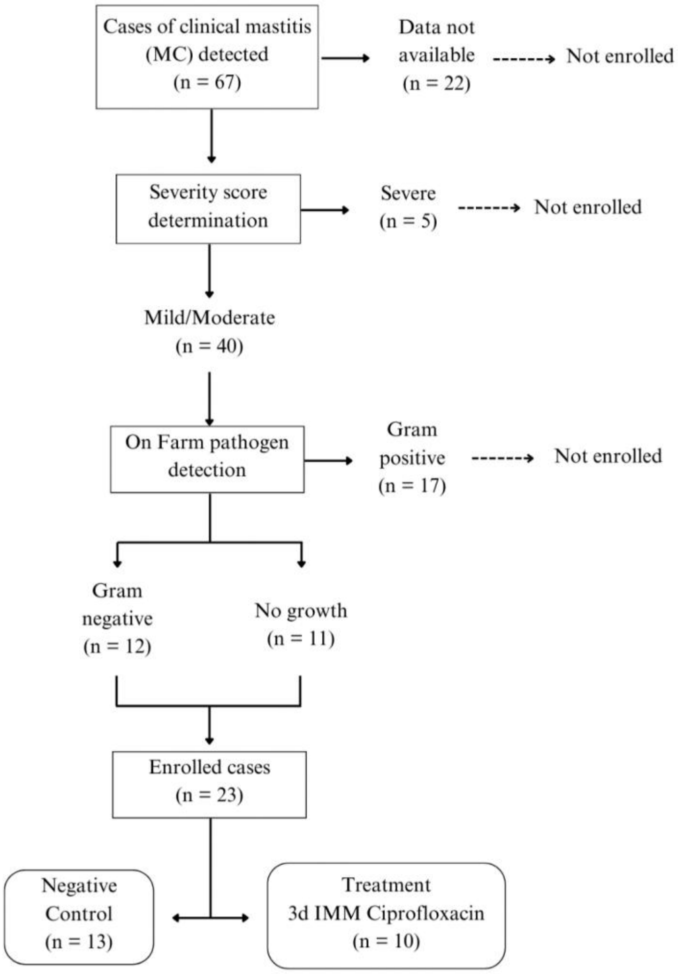

Figure 1. Flow diagram for the enrolled cases of clinical mastitis (CM) in the clinical trial.

Table 1. Description of enrolled cases by experimental group

	Experimental Group		
Item	Negative Control (n=13)	Treated (n=10)	*P*-value[1]
Severity			0.99
Mild	7	5	
Moderate	6	5	
Parity			0.99
2nd	4	3	
≥3rd	9	7	
Pregnancy status			0.99
Yes	4	3	
No	9	7	
DIM			0.99
0-100	6	4	
>100	7	6	
Culture result			0.99
Gram-negative	7	5	
No growth	6	5	

[1]Chi-Square test and Fisher’s Exact test used to compare negative control and treated.

Table 2. Mean ± standard error of rumination, activity, and rest times (min/day) of treated group (3 d treatment with IMM infusion of ciprofloxacin) and negative control (no treatment) of cows diagnosed with mild and moderate clinical mastitis caused by gram-negative bacteria or culture negative

	Experimental Group		*P*-value[1]		
Variable	Negative Control	Treated	Group	Day	G*D[2]
Rumination (min/day)	642.2 ± 16,8	631.9 ± 20,2	0.70	0.16	0.18
Activity (min/day)	186.9 ± 14,5	209.9 ± 17,5	0.32	0.24	0.68
Rest (min/day)	608.6 ± 25,8	595.4 ± 31,0	0.74	0.11	0.18

[1]Mixed ANOVA included parity and DIM at enrollment as covariates.
[2]Group*Day interaction.

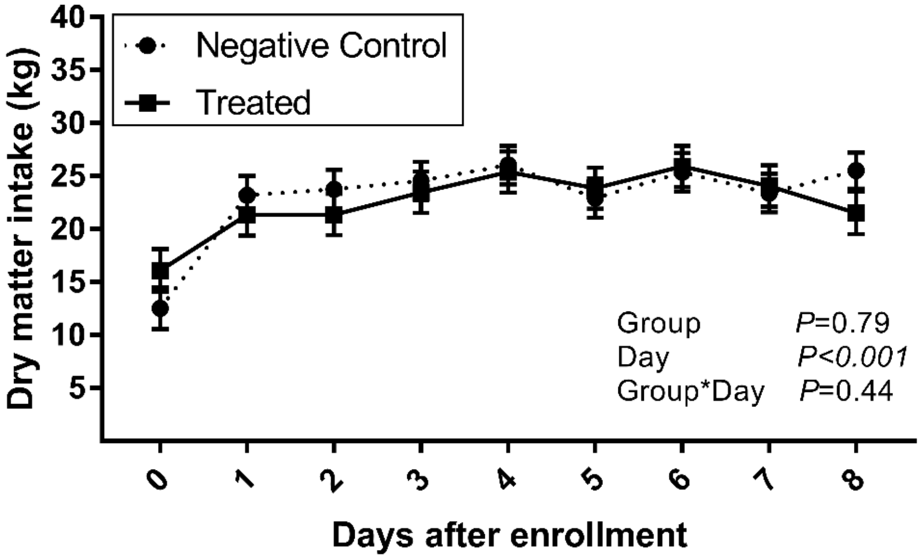

Figure 2. Average dry matter intake from d0 to d8 relative to case enrollment of treated group (n = 10) and negative control (n = 13) of cows diagnosed with mild and moderate clinical mastitis caused by gram-negative bacteria or culture negative. Treatment consisted of a daily IMM infusion of ciprofloxacin (Ciprolac, Ouro Fino Saúde Animal, Brazil) for three consecutive days. Error bars indicate the standard error.

Table 3. Mean ± standard error of feeding behavior of treated group and negative control of cows diagnosed with mild and moderate clinical mastitis caused by gram-negative bacteria or culture negative

	Experimental Group		*P*-value[1]		
Variable	Negative Control	Treated	Group	Day	G*D[2]
FT[3] (min/day)	174.6±9.6	166.2±10.7	0.56	<0.001	0.89
NVF[4]	50.3±3.6	53.9±4.1	0.50	<0.001	0.83
NVFC[5]	33.6±3.0	37.5±3.4	0.40	0.002	0.68

[1]Mixed ANOVA included parity and DIM at enrollment as covariates.
[2]Group*Day interaction.
[3]Feeding time.
[4]Number of visits to the feeder.
[5]Number of visits to the feeder with consumption.

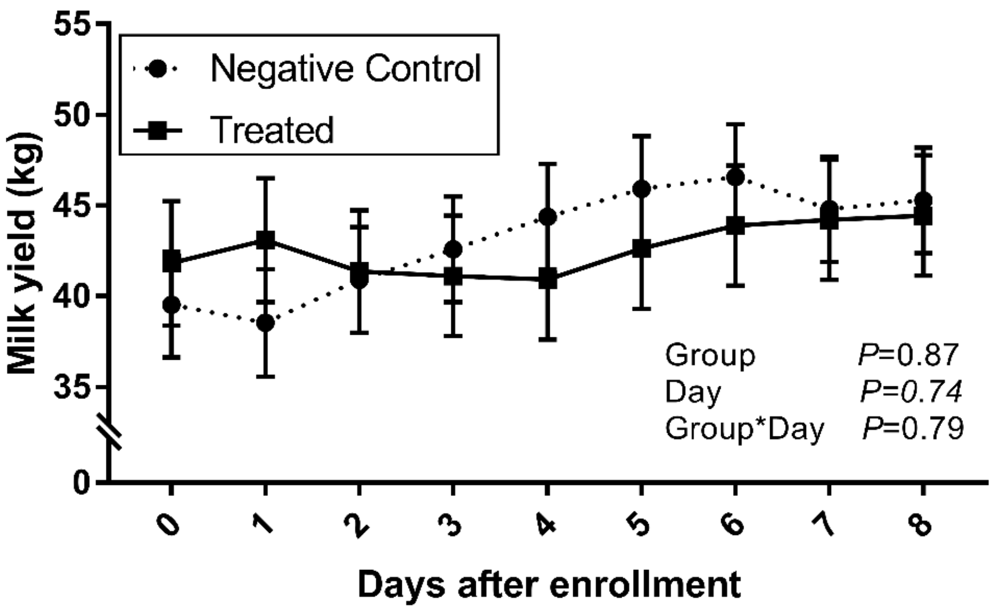

Figure. 3. Average daily milk yield from d0 to d8 in relation to case enrollment of treated group (n = 10) and negative control (n = 13) of cows diagnosed with mild and moderate clinical mastitis caused by gram-negative bacteria or culture negative. Treatment consisted of a daily IMM infusion of ciprofloxacin (Ciprolac, Ouro Fino Saúde Animal, Brazil) for three consecutive days. Error bars indicate the standard error.

DISCUSSION

The study design chosen was a negatively controlled randomized clinical trial, which is considered the gold standard for determining the efficacy of a therapy, comparing it with the immune system response, thus it is possible to observe if there is any benefit added by the treatment (Ruegg, 2021). The IMM antimicrobial used was ciprofloxacin, a second-generation quinolone which has been already demonstrated as effective against gram-negative bacteria (Sharma *et al*., 2010). Clinical cure was observed for all enrolled cases within the observation period of 9 days, which is in accordance with other studies that found an average time of 7 days until clinical cure (Pinzón-Sánchez and Ruegg, 2011; Fuenzalida and Ruegg, 2019a, 2019b). However, clinical cure by itself is not a good indicator of effectiveness of a treatment for CM due to the self-limiting nature of this disease (Ruegg, 2021).

Mastitis, like other diseases, has a negative impact on animal welfare, partially due to the release of pro-inflammatory cytokines and due to the pain originated from the inflammatory response (Fogsgaard *et al*., 2012; Kuhla, 2020). This impact can be observed on the behavior and feed intake, comparing healthy animals and the ones affected by a disorder. A common observed behavioral change is decreased feed intake, activity level, and rumination time, detected few days before the onset of the clinical signs (Fogsgaard *et al*., 2015). The current study was the first negatively controlled clinical trial to assess behavior and feed intake of gram-negative or culture-negative mild and moderate cases of naturally occurring CM, aiming to understand if an IMM antibiotic treatment could bring benefits to the animals compared with the immune response itself.

Recent studies showed that rumination and activity are affected in cows with CM. Stangaferro *et al*. (2016), evaluated if a health-monitoring system, which computes times of rumination and activity, could detect cows with mastitis, found greater sensibility for severe cases of CM, particularly caused by *E. coli*. In experimentally induced mastitis, rumination was lower when cows had severely swollen quarters and pyrexia (Siivonen *et al*., 2011). In a previous study, conducted on the same dairy farm where the present clinical trial was performed, Schmidt *et al*. (2021) observed a reduction of almost 15% in rumination time in cows diagnosed with CM, compared with a period of 15 days prior to the cases detection, without any other disease involved. In that study all levels of severity were included, thus the inclusion of severe cases of CM could have been responsible for the lower average of rumination time observed.

The present clinical trial found no differences in rumination, activity, and rest times, which remained fairly steady throughout the entire studied period, between treated cases and negative control. Medrano-Galarza *et al*. (2012), also found no effect of an IMM antibiotic on behavior (lying time, laterality of lying and restless behavior during milking) in an experiment conducted with healthy nonmastitic cows. Moreover, other two studies observed no effect of different anti-inflammatory drugs on daily rumination time in experimentally induced mastitis (Fitzpatrick *et al*., 2013; Chapinal *et al*., 2014).

Perhaps the changes observed in behavior are influenced by the severity of mastitis and non-severe cases of CM may have no significant impact on rumination, activity, and rest times, which are considered as core maintenance behavior, the last to change in diseased animals (Caplen and Held, 2021). Therefore, there is no sense in treating mild and moderate cases of CM with IMM antimicrobials to prevent changes in behavior, since it seems that such cases do not produce these changes.

Dry matter intake and feeding behavior were not different between the experimental groups during the follow up period. However, there was a significant increase in these parameters one day after the case detection, remaining constant afterwards. Sepúlveda-Varas *et al*. (2016) found a reduction in feed intake, feeding rate, number of visits to the feeder, and number of replacements (competitive behavior) in cows with moderate CM (including animals with fever), beginning five days before clinical signs were evident. The same authors found an increase in those parameters one day after diagnosis which corresponded to the second day of treatment.

Previous studies have been conducted to assess milk yield in cows diagnosed with CM, comparing the selective treatment (antibiotics are given only in mild or moderate cases of CM caused by gram-negative bacteria or with no growth in culture media) with the blank therapy strategy (all cases of CM are treated with antimicrobials), and they found no difference between these two strategies, showing that the antimicrobial failed to improve milk production for the referred cases of mastitis (Lago *et al*., 2011b; Vasquez *et al*., 2017). Despite the shorter follow-up period, the present results agree with previous studies, since there was no difference in the average daily milk yield between negative control and treated cases. Thus, our results add to the recent findings, supporting the idea that IMM antibiotic treatment does not improve milk production in non-severe gram-negative or culture negative cases of CM.

Another important consequence of mastitis is the increase in SCC (Cheng and Han, 2020). During the IMI there is a fast influx of neutrophils from the bloodstream to the mammary gland to eliminate the bacterial infection (Burvenich *et al*., 1994). As soon as the invading pathogen is destroyed, leukocyte recruitment stops and a decrease in SCC is observed a few days after the beginning of the infection, since neutrophils last only few days in the tissues (Sordillo *et al*., 1997). No difference was found in SCC between the experimental groups, yet there was a time effect, where animals from the negative control showed a significant reduction in SCC, while the treatment group had a trend towards reduction. If the analysis of SCC were performed a few days later, perhaps the effect would be observed in the treated animals as well.

Studies comparing the WBC count of healthy animals and animals with CM, observed that affected animals have an increase in WBC count, which could exceed 17,000 cells/µL (Alhussien *et al*., 2015; Sadek *et al*., 2017). Unlike SCC, which reduced, an increase in WBC was observed for both groups. Nevertheless, other leukocytes, such as macrophages and lymphocytes, also have their serum levels increased during the immune response, remaining high for a longer time, once they have a slower action compared with neutrophils (Halasa and Kirkeby, 2020), which may explain the difference observed between SCC and WBC, in the follow up period.

The damage caused by inflammation on the alveolar epithelial cells, responsible for the synthesis of milk components, has a negative impact on milk quality (Auldist and Hubble, 1998). There is a reduction in the concentration of lactose, which is directly linked to the redirection of glucose for the activation of the immune system (Kvidera *et al*., 2017), in addition to a change in the proportion of proteins. In the present study, there was no difference in the milk concentrations of protein, fat and lactose between negative control and treated cases, demonstrating that the IMM ciprofloxacin therapy did not bring benefits to milk quality.

CONCLUSION

Our study focused on the behavior of the animals, and it was able to verify that there were no differences between treated and non-treated animals, which reinforces the results from previous studies showing that mild and moderate CM cases caused by gram-negative pathogens or culture negative do not benefit from IMM antibiotic treatment. The selective treatment of CM is a promising therapeutic strategy which is aligned to the one health concept. The adoption of such a strategy has the potential to reduce antibiotics usage and therefore decrease the risks associated with antimicrobial resistance. Moreover, costs with medicines and discarded milk can be reduced, which will improve the financial sustainability of dairy farms.

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REFERENCES

AJOSE, D.J.; OLUWARINDE, B.O.; ABOLARINWA, T.O. *et al*. Combating bovine mastitis in the dairy sector in an era of antimicrobial resistance: ethno-veterinary medicinal option as a viable alternative approach. *Front. Vet. Sci.*, v.9, p.800322, 2022.

ALHUSSIEN, M.; KAUR, M.; MANJARI, P. *et al*. A comparative study on the blood and milk cell counts of healthy, subclinical, and clinical mastitis Karan Fries cows. *Vet. World*., v.8, p.685-689, 2015.

AULDIST, M.J.; HUBBLE, I.B. Effects of mastitis on raw milk and dairy products. *Aust. J. Dairy Technol.*, v.53, p.28-36, 1998.

BURVENICH, C.; PAAPE, M.J.; HILL, A.W. Role of the neutrophil leucocyte in the local and systemic reactions during experimentally induced e.coli mastitis in cows immediately after calving. *Vet. Q*., v.16, p.45-50, 1994.

CAMPOS, J.L.; KATES, A.; STEINBERGER, A. *et al*. Quantification of antimicrobial usage in adult cows and preweaned calves on 40 large Wisconsin dairy farms using dose-based and mass-based metrics. *J. Dairy Sci*., v.104, p.4727-4745, 2021.

CAPLEN, G.; HELD, S.D.E. Changes in social and feeding behaviors, activity, and salivary serum amyloid A in cows with subclinical mastitis. *J. Dairy Sci*., v.104, p.10991-11008, 2021.

CHAPINAL, N.; FITZPATRICK, C.E.; LESLIE, K.E. *et al*. Short communication: automated assessment of the effect of flunixin meglumine on rumination in dairy cows with endotoxin-induced mastitis. *Can. J. Anim. Sci.*, v.94, p.21-25, 2014.

CHENG, W.N.; HAN, S.G. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments — a review. *Asian Australas. J. Anim. Sci*., v.33, p.1699-1713, 2020.

FITZPATRICK, C.E.; CHAPINAL, N.; PETERSSON-WOLFE, C.S. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. *J. Dairy Sci.*, v.96, p.2847-2856, 2013.

FOGSGAARD, K.K.; BENNEDSGAARD, T.W.; HERSKIN, M.S. Behavioral changes in freestall-housed dairy cows with naturally occurring clinical mastitis. *J. Dairy Sci.*, v.98, p.1730-1738, 2015.

FOGSGAARD, K.K.; RØNTVED, C.M.; SØRENSEN, P. *et al*. Sickness behavior in dairy cows during Escherichia coli mastitis. *J. Dairy Sci*., v.95, p.630-638, 2012.

FUENZALIDA, M.J.; RUEGG, P.L. Negatively controlled, randomized clinical trial to evaluate use of intramammary ceftiofur for treatment of nonsevere culture-negative clinical mastitis. *J. Dairy Sci*., v.102, p.3321-3338, 2019a.

FUENZALIDA, M.J.; RUEGG, P.L. Negatively controlled, randomized clinical trial to evaluate intramammary treatment of nonsevere, gram-negative clinical mastitis. *J. Dairy Sci.*, v.102, p.5438-5457, 2019b.

GONÇALVES, J. L.; FREU, G.; GARCIA, B.L.N. *et al*. Effect of bovine subclinical mastitis on milk production and economic performance of Brazilian dairy farms. *Braz. J. Vet. Res. Anim. Sci*., v.60, p.e208514, 2023.

GRANJA, B.M.; FIDELIS, C.E.; GARCIA, B.L.N. *et al*. Evaluation of chromogenic culture media for rapid identification of microorganisms isolated from cows with clinical and subclinical mastitis. *J. Dairy Sci*., v.104, p.9115-9129, 2021.

GUIMARÃES, J.L.B.; BRITO, M.A.V.P.; LANGE, C.C. *et al*. Estimate of the economic impact of mastitis: a case study in a Holstein dairy herd under tropical conditions. *Prev. Vet. Med.*, v.142, p.46-50, 2017.

GUZMÁN-LUNA, P.; NAG, R.; MARTÍNEZ, I. *et al*. Quantifying current and future raw milk losses due to bovine mastitis on European dairy farms under climate change scenarios. *Sci. Total Environ.*, v.833, p.155149, 2022.

HALASA, T.; KIRKEBY, C. Differential somatic cell count: value for udder health management. *Front. Vet. Sci*., v.7, p.609055, 2020.

KUHLA, B. Review: pro-inflammatory cytokines and hypothalamic inflammation: Implications for insufficient feed intake of transition dairy cows. *Animal*, p.S65-S77, 2020.

KVIDERA, S.K.; HORST, E.A.; ABUAJAMIEH, M. *et al*. Glucose requirements of an activated immune system in lactating Holstein cows. *J. Dairy Sci*., v.100, p.2360-2374, 2017.

LAGO, A.; GODDEN, S.M.; BEY, R. *et al*. The selective treatment of clinical mastitis based on on-farm culture results: 1. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. *J. Dairy Sci*., v.94, p.4441-4456, 2011a.

LAGO, A.; GODDEN, S.M.; BEY, R. *et al*. The selective treatment of clinical mastitis based on on-farm culture results: 2. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. *J. Dairy Sci*., v.94, p.4457-4467, 2011b.

MEDRANO-GALARZA, C.; GIBBONS, J.; WAGNER, S. *et al*. Behavioral changes in dairy cows with mastitis. *J. Dairy Sci*., v.95, p.6994-7002, 2012.

MORALES-UBALDO, A.L.; RIVERO-PEREZ, N.; VALLADARES-CARRANZA, B. *et al*. Bovine mastitis, a worldwide impact disease: prevalence, antimicrobial resistance, and viable alternative approaches. *Vet. Anim. Sci.*, v. 21, p.100306, 2023.

NRC, In. "Nutrient requirements of dairy cattle." National Research Council, Washington, DC, 2001.

OLIVEIRA, C.S.F.; HOGEVEEN, H.; BOTELHO, A.M. *et al*. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. *Prev. Vet. Med*., v.121, p.297-305, 2015.

PINZÓN-SÁNCHEZ, C.; CABRERA, V.E.; RUEGG, P.L. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. *J. Dairy Sci*., v.94, p.1873-1892, 2011.

PINZÓN-SÁNCHEZ, C.; RUEGG, P.L. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. *J. Dairy Sci*., v.94, p.3397-3410, 2011.

POL, M.; RUEGG, P.L. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. *J. Dairy Sci*., v.90, p.249-261, 2007.

PYÖRÄLÄ, S.; KAARTINEN, L.; KÄCK, H. *et al*. Efficacy of two therapy regimens for treatment of experimentally induced Escherichia coli mastitis in cows. *J. Dairy Sci*., v.77, p.453-461, 1994.

ROLLIN, E.; DHUYVETTER, K.C.; OVERTON, M.W. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. *Prev. Vet. Med*., v.122, p.257-264, 2015.

ROMERO, J.; BENAVIDES, E.; MEZA, C. Assessing financial impacts of subclinical mastitis on Colombian Dairy Farms. *Frontiers in Veterinary Science*, v.5, 2018.

RUEGG, P.L. A 100-Year Review: Mastitis detection, management, and prevention. *J. Dairy Sci.*, v. 100, p.10381-10397, 2017.

RUEGG, P.L. What is success? A narrative review of research evaluating outcomes of antibiotics used for treatment of clinical mastitis. *Front. Vet. Sci*., v.8, p.639641, 2021.

SADEK, K.; SALEH, E.; AYOUB, M. Selective, reliable blood and milk bio-markers for diagnosing clinical and subclinical bovine mastitis. *Trop. Anim. Health Prod*., v.49, p.431-437, 2017.

SCHMIDT, A.P.; VIEIRA, L.V.; BARBOSA, A.A. *et al*. Use of the rumination profile through collar sensors for mastitis diagnosis in dairy cows. *Acta Sci. Vet*., v.49, 2021.

SEPÚLVEDA-VARAS, P.; PROUDFOOT, K.L.; WEARY, D.M. *et al*. Changes in behaviour of dairy cows with clinical mastitis. *Appl. Anim. Behav. Sci*., v.175, p.8-13, 2016.

SHARMA, P.C.; JAIN, A.; JAIN, S. *et al*. Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects. *J. Enzyme Inhib. Med. Chem*., v.25, p.577-589, 2010.

SHARUN, K.; DHAMA, K.; TIWARI, R. *et al*. Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. *Vet. Q*., v.41, p.107-136, 2021.

SIIVONEN, J.; TAPONEN, S.; HOVINEN, M. *et al*. Impact of acute clinical mastitis on cow behaviour. *Appl. Anim. Behav. Sci*., v.132, p.101-106, 2011.

SORDILLO, L.M.; SHAFER-WEAVER, K.; DEROSA, D. Immunobiology of the mammary gland. *J. Dairy Sc*i., v.80, p.1851-1865, 1997.

STANGAFERRO, M.L.; WIJMA, R.; CAIXETA, L.S. *et al*. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part 2. Mastitis. *J. Dairy Sci*., v.99, p.7411-7421, 2016.

VASQUEZ, A.K.; NYDAM, D.V.; CAPEL, M.B. *et al*. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. *J. Dairy Sci*., v.100, p.2992-3003, 2017.