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Effectiveness of HoofCare® in the treatment of digital dermatitis in dairy cows

[*Eficácia do HoofCare® no tratamento da dermatite digital em vacas leiteiras*]

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ABSTRACT

This study aimed to determine the effectiveness of topical HoofCare® in the treatment of digital dermatitis (DD) in dairy cows. Thirty-six high-yielding Holstein cows with DD in at least one limb were included and randomly divided into two groups, making 42 limbs. The alternating treatment group consisted of 21 limbs treated for three alternating days every 48h, and the continuous treatment group consisted of 21 limbs treated every 24h for five days. The cows underwent lameness examination, thermographic analysis, and qualitative and quantitative analysis of the lesions on D0 (time of identification of the lesion and prior to treatment), D4 (fourth day of treatment), and D10 (five days after the end of treatment). The data were compared between different time points and groups. There was no significant difference between the groups for the variables evaluated. However, both groups presented a reduction in the degree of lameness around the lesion, in the local temperature, and regression from the active to the inactive stage of the lesions. Thus, this short-term study demonstrates the suggestive efficacy of HoofCare® in treating DD and recommends conducting further long-term studies.

Keywords: bovine digital dermatitis, dairy cows, thermography, clinical evolution

RESUMO

Este estudo teve como objetivo determinar a eficácia do HoofCare® tópico no tratamento da dermatite digital (DD) em vacas leiteiras. Trinta e seis vacas Holandesas de alto rendimento, com DD em pelo menos um dos membros, foram incluídas e divididas aleatoriamente em dois grupos, totalizando 42 membros. O grupo de tratamento alternado consistiu de 21 membros tratados por três dias alternados, a cada 48 horas, e o grupo de tratamento contínuo consistiu de 21 membros tratados a cada 24 horas, por cinco dias. As vacas foram submetidas ao exame de claudicação, à análise termográfica, à análise qualitativa e quantitativa das lesões no D0 (momento da identificação da lesão e antes do tratamento), no D4 (quarto dia de tratamento) e no D10 (cinco dias após o término do tratamento). Os dados foram comparados entre diferentes momentos e grupos. Não houve diferença significativa entre os grupos para as variáveis avaliadas. Entretanto, ambos os grupos apresentaram redução no grau de claudicação, na área da lesão, na temperatura local e regressão do estágio ativo para o inativo das lesões. Assim, este estudo de curto prazo demonstra sugestiva eficácia do HoofCare® no tratamento da DD e recomenda a realização de mais estudos de longo prazo.

Palavras-chave: dermatite digital bovina, vacas leiteiras, termografia, evolução clínica

INTRODUCTION

Digital dermatitis (DD) is one of the most prevalent foot diseases in dairy cattle (Cruz *et al.*, 2001; Borges *et al.*, 2017), inflicting losses to livestock because it increases costs with treatments, milk disposal, and decreased zootechnical performance (Cha *et al.*, 2010; Akin and Akin, 2018). This disease is currently endemic in many parts of the world (Wilson-Welder *et al.*, 2015), with its prevalence ranging from 3.32% (Ahmed and Shekidef, 2012) to 83% (Holzhauer *et al.*, 2006).

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DD is a highly contagious infectious disease that causes pain, decreases in production, as well as increases in reproductive problems and susceptibility to other diseases (Berry *et al*., 2012; Refaai *et al*., 2013; Nielsen *et al*., 2016; Pozzatti *et al*., 2018), even in cases without apparent lameness (Laven and Proven, 2000). It has a multifactorial etiology with several predisposing factors such as genetics, diet, management, environment, and preventive trimming (Silva *et al*., 2001; Nishikawa and Taguchi, 2008). Although its etiology has not been fully elucidated, the disease has a polymicrobial character, with the confirmed involvement of *Treponema* spp. (Moreira *et al.*, 2018).

DD is diagnosed by visualization of characteristic ulcerative, erosive, and proliferative lesions (Nielsen *et al*., 2016). Treatment commonly involves antibiotic therapy (Laven and Logue, 2006), and oxytetracycline is the most used antibiotic (Manske *et al*., 2002; Silva *et al*., 2005; Loureiro *et al*., 2010; Cramer *et al*., 2019).

The therapeutic options for the disease are limited, with the possible presence of strains resistant to some conventional antibiotic treatments (Shearer and Hernandez, 2000; Kamiloglu *et al*., 2002; Nishikawa and Taguchi, 2008), which may be associated with the presence of antibiotic residues in milk and the need to respect the withdrawal period for its consumption (Cunha, 2000; Cramer *et al*., 2019). In addition, the demand for organic milk has been significantly growing, suggesting that the production of good quality milk is no longer a choice but a necessity (Soares *et al*., 2011).

The use of antibiotic-free therapies is becoming increasingly common, with studies suggesting good results using copper sulfate (Teixeira *et al*., 2010), extract from trees of the genus *Copaifera reticulata* (Copaiba oil) (Bomjardim *et al*., 2020), salicylic acid (Bomjardim *et al*. 2020, Schultz and Capion, 2013), and photodynamic phototherapy (Sellera *et al*., 2021). A product made from citric acid, tea tree essential oil, copper citrate, and zinc chloride has been used in several Brazilian farms with good results in treating DD. However, these are still empirical field observations, with no scientific information about the real effectiveness of the product and the best treatment protocol. This study aimed to determine the effectiveness of brush stroke an antibiotic-free gel specific for claws in the treatment of DD in dairy cattle, as well as analyze if there is superior effectiveness of the continuous protocol to the alternating one.

MATERIALS AND METHODS

The present study was approved by Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science, Paulista State University (CEUA/UNESP protocol n. 169/2020). Thirty-six Holstein lactating cows aged between two and ten years from a commercial dairy farm in the municipality of Anta Gorda, Rio Grande do Sul, Brazil were included in this study. These animals were housed in a compost barn system with a sawdust bed and automatic fans separated into batches by category. They were milked thrice daily and received a balanced diet (commercial feed, soybean hulls, cottonseed, silage, and pre-dried ryegrass). The inclusion criterion was the presence of DD in at least one of the limbs, and the exclusion criterion was the presence of other concurrent disorders and lesion claws.

The farm did not use preventive measures against the disease, such as footbaths and quarantine for newly acquired animals, but had veterinary assistance specialized in bovine podiatry at least once a year. The cows were kept under natural conditions at the farm, with no diet and management changes during the experiment.

Prior to instituting treatment, all cows were identified by their farm tag and examined. Relevant information such as age, category, mean milk production/day, calving number, and the injured limb was recorded on an evaluation form. The animals were diagnosed by a veterinarian specializing in bovine podiatry while contained at the time of routine trimming. The digit was examined after the limb was suspended, trimmed, and sanitized (Dirksen, 1993). Limbs with active DD lesions, i.e., with pain and increased temperature on palpation, were included in the study. After the clinical diagnosis of the disease, 36 animals were assessed for the locomotion score and DD lesion severity, according to Kofler *et al*. (2020).

The limbs with DD were randomly divided into two treatment groups soon after the diagnosis, regardless of lesion severity, in order to produce representative disease units in each group; The first limb was assigned to the alternating treatment group (ATG), the second one was assigned to the continuous treatment group (CTG), and so forth, always alternating the affected limb. If the cow had more than one injured limb, the second limb was assigned to the next treatment group. Animals in the ATG had a green cotton rope, and animals in the CTG had a blue cotton rope on the neck and on the lesioned limb in order to differentiate the groups.

Both groups were treated by brush stroke with HoofCare® (HoofCare® - citric acid, tea tree essential oil, copper citrate, polysaccharide, bright blue dye, and zinc chloride – Salmix), a specific antibiotic-free gel for the hoof, composed of antimicrobials and healing agents (copper, zinc, and tea tree essential oil). The ATG consisted of 21 limbs (18 cows) treated every 48 hours for five days, and the CTG consisted of 21 limbs (18 cows) treated for five consecutive days, totaling 42 members.

There was no control group, just two groups of different treatments (alternating and continuous), since so far, spontaneous DD wound healing rarely happens, with lesions remaining at the same stage for two years or more (Krull *et al*., 2014). Although simple cleaning with water can promote a reduction in the prevalence of DD (Thomsen *et al*., 2008), and even a cure (Manske *et al*., 2002), this methodology was chosen because the study was carried out on a high-production commercial farm. The disease has a great economic impact, being considered the most expensive foot disease (US$95 per case), with losses estimated at US$126 to $133 for each clinical case (Cha *et al*., 2010), especially in milk production, with difference significant difference in kg/day of milk before treatment and after treatment (Akin and Akin, 2018), which in financial terms it would not be viable for the farm. Due to the highly contagious nature of DD, the possibility of rapid dissemination in the herd is high, since lesions in the active stage (M2) can persist for a long period of time (Somers *et al*., 2005b) and chronically infected animals can act as reservoirs and a potential source of outbreaks (Solano *et al*., 2017). Furthermore, in some cases the negative control with only saline solution resulted in the progression of the lesions (Stevancevic *et al*., 2009), suggesting the complexity of the etiology of DD, as well as the variation in susceptibility between animals (Capion *et al*., 2013). Therefore, as the farm does not have prevention measures, it was preferred to use the moment prior to treatment (D0) as a control for the other moments, that is, the animal being its own control.

Thermographic analysis of the lesion, lameness examination, and photography of the lesions for further analyses were performed at three points to determine clinical recovery and full recovery. The three daily milking procedures were routinely maintained at the farm, with the place being cleaned daily at the end of each milking period.

The lameness test was performed before the animal was restrained in the chute to evaluate the clinical effectiveness of the product in treating DD and determine the locomotion score, according to Thomsen *et al*. (2008). The score was assessed at three-time points (D0, D4, and D10). After the end of data collection, the animals were evaluated to investigate whether there was an improvement, worsening, or if the score remained the same.

The animals were contained in a chute adapted for trimming and kept in a quadrupedal position without the need for tranquilization, according to what was described by Loureiro *et al*. (2010). The limb to be treated was elevated and restrained. Subsequently, it was trimmed and cleaned with running water to remove dirt and ensure better lesion visualization. In cases where more than one DD lesion was observed in the limb, the most severe lesion was selected. All lesions presented pain and increased temperature on palpation before treatment as an inclusion factor (Somers *et al*., 2005a), with animals that presented other concomitant conditions excluded from the study.

The clean lesions were photographed next to a ruler for further classification and morphometry. Six experienced evaluators who were blinded to the time of evaluation and therapeutic protocol used classified the DD. Based on the signs and extent of the affected region, each evaluator classified the 42 lesions into M-stages at D0, D4, and D10, according to the M-scoring system

(Kofler *et al*., 2020). If different DD stages were categorized in the same lesion by the same evaluator the most active prominent stage was set to describe the lesion, i.e., the final score (M2 > M4.1 > M1 > M4 > M3) (Solano *et al*., 2017).

Lesion morphometry is a quantitative assessment of the DD healing process. The pictures at D0, D4, and D10 were analyzed using the ImageJ (ImageJ ®., Java version 6.0 image editing, processing, and analysis program) free image analysis software. As the lesions were photographed next to a ruler, it was possible to create a scale in the software, regardless of the distance between the lesion and the camera. Therefore, first, the lesion areas were measured three times, and the mean dimension in cm² of each lesion was used to compare the time points and the two therapeutic protocols to estimate the mean lesion regression.

Thermographic images were collected using a FLIR Systems (FLIR Systems Inc ®., FLIR SC660 Thermal Imaging) camera before cleaning the claws and identifying the lesions, similar to what was described by Rodrigues (2013), however, with the limb suspended and the cow contained. There was no dirt control before the test, with studies showing no interference (Stokes *et al*., 2012, Harris-Bridge *et al*., 2018). The camera mentioned above has a temperature range of 16.1 ^oC to 28.8^oC and was directed to the lesion region on the evaluated limb at 1m. The image was captured with the animal contained in a chute adapted for trimming and with the limb elevated. As environmental conditions influence the temperature detected by the thermographic camera, the ambient temperature and the relative humidity of the air were monitored during each image capture, according to Harris-Bridge *et al*. (2018).

All assessments were performed after milking at the same time in the afternoon at D0, D4, and D10, and we used the same distance and background. At the end of the experiment, the images were analyzed using the FLIR Systems QuickReport software, which corrects ambient temperature, relative air humidity, and emissivity degree (0.98). The maximum temperatures were subsequently standardized based on the international methodology for atmospheric temperature standardization (Basile *et al*., 2010).

The cows received direct administration of a 5mm layer of HoofCare® along the entire lesion using a brush stroke until the area was covered, every 48 h on ATG or every 24h on CTG. The digits were sanitized with running water before using the product in just two moments, D0 and D4. The other applications were in the milking parlor during the afternoon milking, without prior cleaning, and with the animal in the quadrupedal station to simulate the reality of treatment on a commercial farm.

Data on the category and milk production/day of each animal were tabulated in a Microsoft® Excel spreadsheet for descriptive statistical analysis. Data on lameness score and of limbs with DD (classification, morphometry, and thermography of lesions) were tested for variance normality and homogeneity by the Shapiro-Wilk and Levene tests, respectively.

The locomotion score was compared between time points and treatment group using the Mann-Whitney and Friedman tests, respectively. After the classification by the evaluators, the M-stages were ordered into three stages (0 =M0; 1 = M3 and M4; 2 = M1, M2, and M4.1) to classify the lesion as non-existent (0), inactive (1), or active (2). After confirming a non-parametric distribution, the treatments and the time points within each treatment were compared using Mann-Whitney and Friedman tests, respectively. The agreement between evaluators and the ordered outcome was analyzed using the Kappa Fleiss test (k). Results were interpreted according to Landis and Koch (1977) as ≤ 0 = poor; 0.01-0.20 = mild; 0.21-0.40 = regular; 0.41-0.60 = moderate; 0.61-0.80 = substantial; and 0.81-1.00 = almost perfect.

Numerical measurement data (morphometry and thermography) were subjected to analysis of variance (ANOVA) and Tukey’s test to compare the means of the different time points between the two groups. The analyses were performed using Past 4.05 software, and the significance level was set at $P < 0.05$.

RESULTS

Thirty-six Holsteins females with a 3.5 mean age and mean milk production of 40.10kg/day affected with DD were used in this study. In thirty animals, lesions were restricted to one limb affected, and of the 42 limbs with DD, only 14.30% (6/42) were in the forelimbs. Disease prevalence in the lactating herd was estimated at 20% (36/150), 50% of the cows were primiparous, and 50% were multiparous, with no significant difference in age between the two treatment groups.

The locomotion score showed mild lameness (2/5) at the time of lesion identification and prior to treatment, progressing to normal locomotion (1/5) in the last evaluation (Fig. 1). There was a statistical difference between different time points in the two groups ($P < 0.01$), however, there was no significant difference between groups.

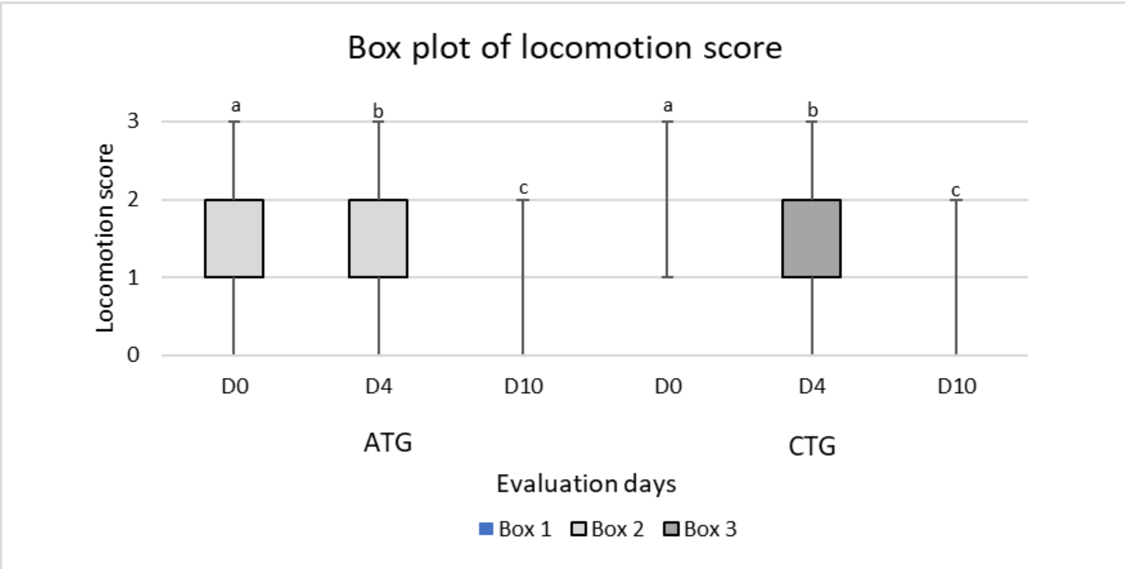

Figure1. Results of the evaluation of the locomotion score of Holstein cows with DD treated with HoofCare® alternately (ATG) and continuously (CTG) over a ten-day period. The box plots show the 25th (box 1) and 75th (box 3) percentiles; bars indicate a CI of 95%. N = 21/group. Different letters between the box plots indicate a significant difference ($P < 0.05$).

Both treatments were effective for lesions regression ($P < 0.01$), as active lesions on D0, were inactive on D10. The non-existent stage was not observed at any time during the study. The test did not show a significant difference between the therapeutic protocols, however, there was a higher percentage of limbs with inactive lesions in the CTG (90.5%) than in the ATG (71.4%) on D10, as shown in Figure 2. Nonetheless, there was a recurrence of 9.5% of the lesions in the CTG and 9.6% in the ATG on D10. The Kappa value among the six evaluators was considered mild on D0 in both groups, non-existent on D4 for the CTG, and regular at other times for both groups.

Lesion morphometry suggested that 100% of the lesions were regressive in the area (Fig. 3). The area medians differed ($P < 0.05$) on D0 and D10 in the ATG, however, there was no significant difference between treatment groups in the medians at different time points or in the mean regression (2.30cm² in the ATG and 2.49cm² in the CTG).

The lesion regression index, i.e., the percentage lesion reduction from D0 to D10, did not differ between the groups ($P = 0.66$). However, the percentage of lesions with an index > 30% was higher in the CTG (13/21, 61.9%) than in the ATG (10/21, 47.6%) (Fig.4).

Table 1 shows the stage of the lesions, the average area of the lesion in the morphometric analysis, and the temperature corrected by the thermography at the three moments evaluated to compare the groups.

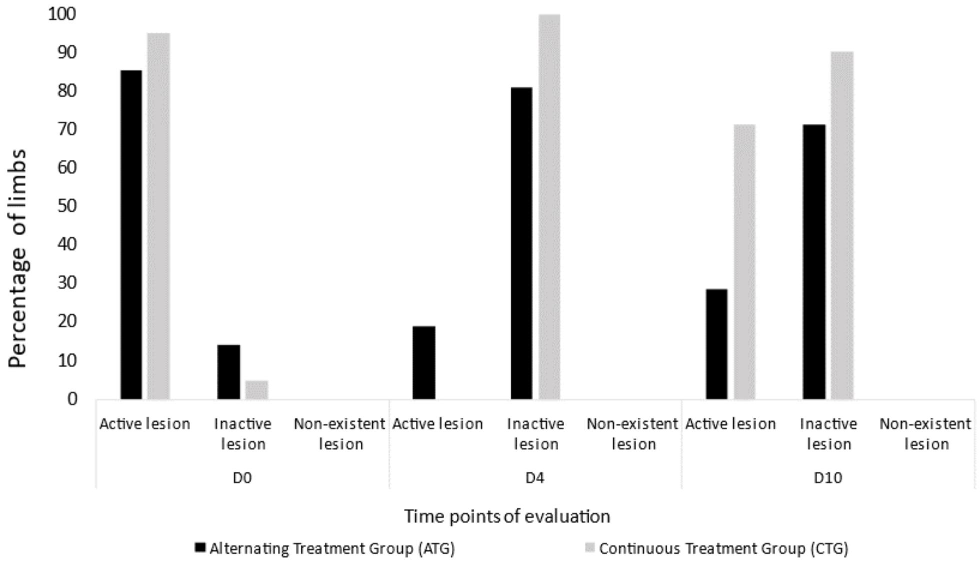

Figure 2. Clinical progression and comparison of the transition from active to inactive stage of 42 lesions DD in 36 Holstein cows undergoing alternating and continuous HoofCare® treatment.

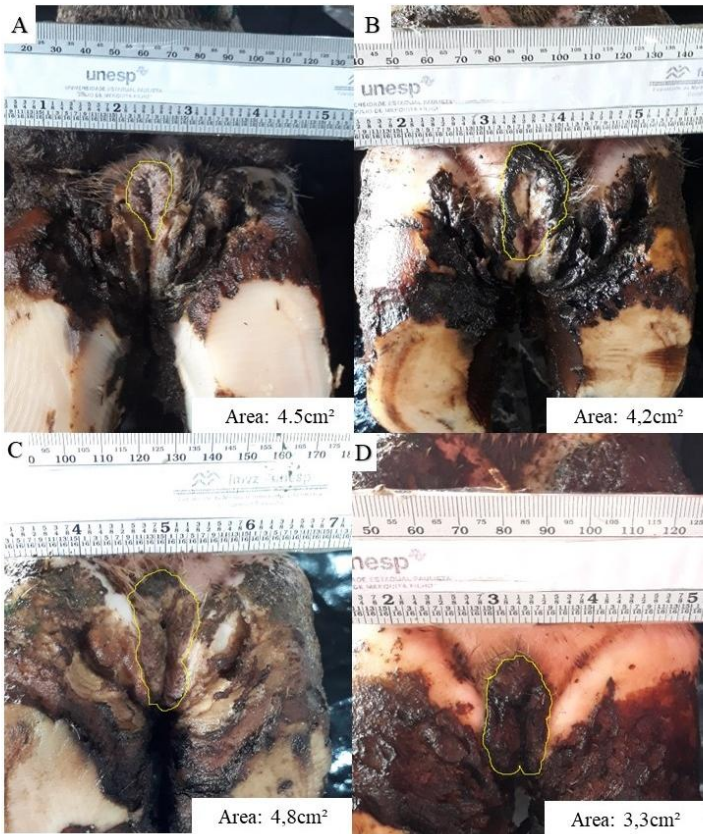

Figure 3. Comparison of DD lesions morphometry in dairy cows submitted to the two HoofCare® treatment protocols. **A.** Before alternating treatment, brush stroke every 48 hours. **B.** Five days after the end of the alternating treatment. **C.** Before continuous treatment, brush stroke every 24 hours**. D**. Five days after the end of continuous treatment.

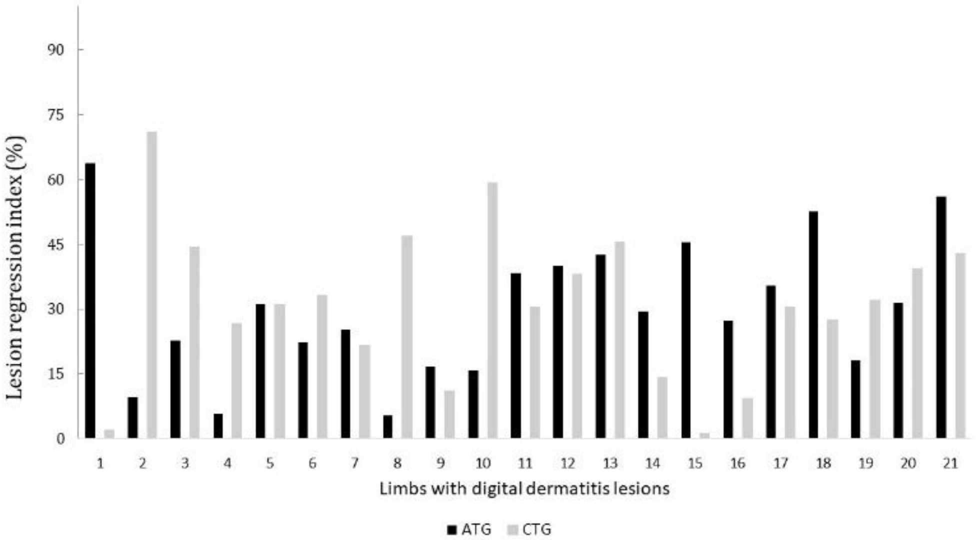

Figure 4. Comparison of the regression indices of DD lesion areas in 42 Holstein cow limbs undergoing alternating (ATG) and continuous (CTG) HoofCare® treatment on D10 (five days after the end of treatment).

Thermographic analysis revealed reduced temperature after the end of the treatment, as it found a higher median lesion area on D0 than on D10 in both groups (ATG: 31.9 °C and 26.9 °C; CTG: 32.1 °C and 24.9 °C). There was a significant difference between D0 and D10 (Fig. 5) in the two groups, with no difference ($P = 0.96$) between groups, with a mean temperature decrease of 6.4 °C in the ATG and of 6 °C in the CTG.

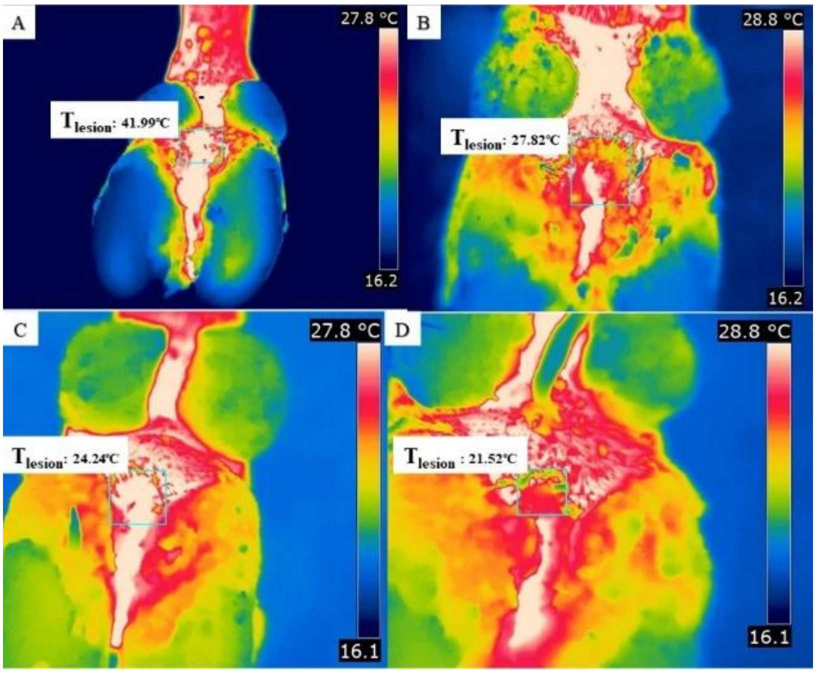

Figure 5. Comparison of the corrected and standardized thermographic temperature of DD lesion areas in dairy cows submitted to the two HoofCare® treatment protocols. **A.** Before the alternate treatment, brush stroke every 48 hours. **B.** Five days after the end of alternate treatment. **C.** Before the continuous treatment, brush stroke every 24 hours. **D.** Five days after the end of continuous treatment.

Table 1. Comparison between the medians [Q1-Q3] of the lesion stages, morphometry, and thermography of dairy cows with DD under alternating and continuous HoofCare® treatment

Evaluated data	Treatment groups	
	ATG (n = 21)	CTG (n = 21)
Lesion stage		
D0	Active [inactive - active] [a]	Active [active - active] [a]
D4	Inactive [inactive - inactive] [b]	Inactive [inactive - inactive] [b]
D10	Inactive [inactive - active] [c]	Inactive [inactive - inactive] [c]
Area of lesion (cm²)		
D0	7.1 [5 – 8.4] [a]	7.6 [5.6 -8.7] [a]
D4	6.2 [4.8 – 70.4] [ab]	6.2 [5.4 – 7] [ab]
D10	4,5 [3.8 – 5.7] [b]	4.7 [3.6 – 5.6] [b]
Lesion temperature (°C)		
D0	31.9 [28.6 – 37.1] [a]	32.1 [28 – 34.8] [a]
D4	19.9 [19.2 – 29.1] [b]	20.3 [18.3-24.4] [b]
D10	26.9 [21.9 – 28.1] [c]	24.9 [22 – 29.9] [c]

(A-B-C) Different letters between lines indicate statistically significant difference (P < 0,05)
(a-b-c) Different letters between columns indicate statistical difference (P < 0,05)

DISCUSSION

The two groups were homogeneous without the need for alterations to reduce interference from external factors (Nielsen *et al*., 2009) since the cows were from the same farm and kept in the same environment and management (Loureiro *et al*., 2010). The evaluations were conducted without difficulty in the chute adapted for trimming, eliminating the need for chemical containment for data collection (Desrochers *et al*., 2001; Loureiro *et al*., 2010).

The lameness test (Thomsen *et al*., 2008) was performed to determine the clinical recovery of DD and, as observed in previous studies (Laven and Proven, 2000; Bomjardim *et al*., 2020), lameness was a discrete clinical sign. Lameness progression throughout the treatment, regressing from mild or moderate to absent, suggests the efficacy of the product in inhibiting disease progression. The mild degree of lameness reinforces the absence of conditions concomitant with DD, such as a sole ulcer or heel erosion, which can worsen lameness (Manske *et al*., 2002; Sellera *et al*., 2018).

The lesions were evaluated and classified by macroscopic appearance into M-stages (Kofler *et al*., 2020) associated with the locomotion score. Classification biases were minimized by the blind categorization of the photographed lesions by evaluators with previous experience with the evaluation method, in contrast to other studies in which classification was performed directly with the animals, associated or not with photographs (Moreira *et al*., 2018; Bomjardim *et al*., 2020). The regular agreement between evaluators confirms the subjectivity of qualitative assessment, justifying the use of several evaluators and the need for prior training to optimize agreement or an unsuitable classification system (Alsaaod *et al*., 2014).

M-stages were classified into three stages (non-existent, inactive, and active lesion) to analyze lesion progression. The significant difference observed between the initial and final DD scores in the two groups indicates that five-day continuous or alternating HoofCare® therapy was effective for lesion regression from active to inactive stages. However, there was a relapse on D10 in both treatment groups, probably due to two causes. First, there was no absence of infection, i.e., the M0 stage was not observed, and therefore, the active infection could be under the crust visualized at M3 and M4 stages (Berry *et al*., 2012; Krull *et al*., 2016; Plummer and Krull, 2017). Compatible with the statement that *Treponema* spp., the main pathogen of DD (Moreira *et al*., 2018), can migrate, penetrate the deep dermis, and form a biofilm, favoring its proliferation and persistence of infection (Beninger *et al*., 2018; Lux *et al*., 2001).

Another possible cause for relapse is reinfection (Plummer and Krull, 2017) since no preventive measures were adopted, and the product was brush stoke on the lesion, with the agent persisting in the environment. In addition, unlike in other studies, complete lesion healing was not observed in any limbs (Elliott *et al*., 2007, Nishikawa and Taguchi, 2008; Holzhauer *et al*., 2011; Krull *et al*., 2016; Moreira *et al*., 2018). This probably happened due to the short assessment time, only five days after treatment, without enough time for total lesion regression, which takes around 42 days (Nielsen *et al*., 2009). Or even for the short treatment period of treatment, requiring a few more days, corroborating the findings of Krull *et al*. (2016), who stated that DD lesions are unlikely to heal with less than seven days of treatment.

The morphometric analysis showed regression with a significantly different lesion area in 100% of cases on D10. This confirmed product effectiveness in both protocols. Moreover, there was a significant regression of lesions in different degrees of DD consistent with the inactive stage observed and mentioned above. Active lesions ranged from 2.2 to 15.4cm², with ulcerative and red or reddish-gray appearance, pain on palpation, and a characteristic odor in some cases. Inactive lesions ranged from 1.3 to 19.2cm², with a grayish to black appearance, hyperkeratosis projections, and no pain on palpation. Some lesions in the inactivation stage increased in diameter, possibly due to the hyperkeratotic projections that may be formed during the healing stage, as observed in a previous study (Bomjardim *et al*., 2020). There was no significant difference in the mean and in the regression index between the proposed therapeutic protocols, with the alternating and continuous treatments showing similar effectiveness. However, CTG showed a higher percentage of lesions (61.9%), with a regression index above 30% of the initial area.

For the exam to be practical, fast, and not need to change the management, we decided to measure the temperature of the DD lesion, unlike previous studies (Alsaaod and Büscher, 2012; Alsaaod *et al*., 2014; Harris-Bridge *et al*., 2018). In addition, the thermography was performed in an uncontrolled environment, without prior removal of dirt and with a suspended limb, contradicting some studies (Nikkhah *et al*., 2005; Basile *et al*., 2010; Alsaaod and Büscher, 2012; Alsaaod *et al*., 2014). Nonetheless, since the methodology was always the same in all animals, not different between groups, there was homogeneity and reliability in the data for comparison. There was no statistical difference in the two protocols evaluated; however, the temperature of the lesion on D10 was lower than the temperature at the initial moment in the ATG (81%) and CTG (90.5%), which supported the other data observed. Although the study methodology was controlled, there was some difficulty in performing the exam without cleaning and drying the limb (Basile *et al*., 2010); however, dirt does not impair the diagnostic accuracy of the exam (Stokes *et al*., 2012; Harris-Bridge *et al*., 2018).

All 36 cows in the study underwent previous trimming, providing a favorable environment for treatment (Manske *et al*., 2002; Toholj *et al*., 2012). Brushing with HoofCare was performed without prior cleaning, which might have influenced the treatment response, as the act of removing dirt improves the contact surface of the lesion with the product (Manske *et al*., 2002; Jacobs *et al*., 2018). However, despite not having been an evaluated variable, the product had good impregnation, even in the presence of organic material. Possibly due to the favorable association of the compounds contained in HoofCare®. Citric acid is a strong stabilizer (Papagianni, 2011; Prastiwi *et al*., 2019); tea tree oil is a potent antiseptic, antimicrobial and anti-inflammatory (Souza *et al*., 2014, Battisti *et al*., 2021; Ramadan *et al*., 2020) and both zinc and copper are essential for tissue repair and potent antimicrobials (Polefka *et al*., 2012; Hobman and Crossman, 2014).

This study had several limitations. First, the post-treatment assessment time may not have been sufficient to confirm the complete healing of the lesions. Therefore, further investigations with a more extended follow-up period are necessary. Second, no preventive measures were adopted in the environment, and the cause of the disease may have persisted during the study.

CONCLUSION

Brushing the HoofCare® over the lesion either continuously or alternately resulted in significantly decreased lameness degree, wound area, and lesion temperature. Thus, this short-term study demonstrates the suggestive efficacy of HoofCare® in inhibiting the progression of DD in the treatment of the disease. However, further long-term studies are recommended in order to prove the complete inactivity of the lesion and its effectiveness in the treatment of DD, as well as the association of control measures.

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