VETERINARSKI ARHIV 86 (2), 197-208, 2016

Comparative therapeutic effect of antiseptic-antibiotic paste for topical treatment of digital dermatitis in dairy cows

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EL-SHAFAEY, E., Y. ELSEADY, S. EL-KHODERY:Comparative therapeutic effect of antiseptic-antibiotic paste for topical treatment of digital dermatitis in dairy cows. Vet. arhiv 86, 197-208, 2016.

ABSTRACT

The present study was designed to evaluate the efficacy of the concomitant use of antiseptic paste with a reduced amount of broad spectrum antibiotic as an alternative topical treatment of digital dermatitis (DD) in dairy cows. Thirty Holstein-Friesian dairy cows with active DD lesions were selected randomly from 93 diseased cases. Cows were randomly allocated for three topical treatment trials (10 each) using antiseptic paste (3 g iodine, 2 g potassium iodine and 30 g zinc oxide/100 g), antibiotic paste (250 mg/ 3gm of cefazolin) and antiseptic-antibiotic paste, under a light bandage for 72 hours. The clinical index scores and case definition scores were assessed on day 0, pre-treatment, and at 7, 14, 21 and 28 days post-treatment. The group treated with antiseptic-antibiotic paste showed significantly decreased scores of lesion depth ($P<0.05$) and size ($P<0.05$) when compared with the groups treated with antiseptic or antibiotic paste on the 21 and 28 days post-treatment. Collectively, the summation of clinical index scores showed a significant decrease ($P<0.05$) in the group treated with the antiseptic-antibiotic combination compared with the antiseptic and antibiotic groups at 14 days post-treatment. By the 28th day, the recovery rates were 9/10, 5/10 and 4/10 for the groups treated with the antiseptic-antibiotic combination, antibiotic or antiseptic paste, respectively. In conclusion, our results suggest the clinically synergistic effect of the topical antiseptic-antibiotic combination, which appears to be an effective alternative treatment for DD in dairy cows, minimizing the amount of antibiotic residues.

Key words: antiseptic-antibiotic paste, case definition, clinical index, cows, digital dermatitis

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ISSN 0372-5480
Printed in Croatia

Introduction

Digital dermatitis (DD) is a global emerging infectious disease, causing lameness in dairy herds, resulting in economic losses in the dairy industry (LAVEN and PROVEN, 2000; MANSKE et al., 2002; REFAAI et al., 2013). Since first being described as a clinical condition in Italy by CHELI and MORTELLARO (1974), it has been detrimental to milk production, reproductive efficiency, productive lifespan and welfare (ETTEMA and ØSTERGAARD, 2006).

DD is manifested by circumscribed lesions on the skin of the claw, mostly between the heels of the hind claws (READ and WALKER, 1998). The precise cause and factors, which predispose to DD occurrence in herds, have not been fully determined, but it appears to be a multifactorial disease involving the Treponema species (GOMEZ et al., 2014) along with environmental (READ and WALKER, 1998), farm-management (SOMERS et al., 2005) and individual animal factors (REFAAI et al., 2013).

Reducing DD prevalence through early detection and treatment is an essential management tool (READ and WALKER, 1998; LAVEN and LOGUE, 2006; RELUN et al., 2013; ALSAAOD et al., 2014). Several chemical compounds have been used in treatment of DD, either in systemic form, individual topical treatment, or mass topical therapy using a footbath (LAVEN and PROVEN, 2000; MOORE et al., 2001; RELUN et al., 2012). Antibiotics are effective against digital dermatitis (BERRY et al., 2010), but are expensive and involve the risk of resistance and residue development. Therefore, alternatives to reduce quantities of antibiotic are urgently needed worldwide (KOFLER et al., 2004). Recently, new trials have been introduced, for the use of antiseptic or antiseptic-antibiotic combinations for topical treatment of DD, with or without a bandage, which have shown comparable efficiency in DD treatment (HOLZHAUER et al., 2011; CUTLER et al., 2013).

There are limited universally recognized alternatives for antibiotic treatment of DD in dairy cows. Therefore, the present study was designed to evaluate the efficacy of the concomitant use of antiseptic paste with a reduced amount of broad spectrum antibiotic, as an alternative treatment of DD in dairy cows.

Materials and methods

Animals and housing. Thirty cows, randomly selected from 93 cows with DD, were enrolled for a randomized clinical trial from January to December 2013. The cows were raised in a commercial Holstein-Friesian dairy herd, with approximately 450 dairy cows. The animals were 2-5 years of age and 400-550 kg of weight. The animals were housed in a cubicle (free-stall/feedlot) barn with straw-bedded stalls, and a slatted floor, which was regularly scraped. The cows were fed a total mixed ration (TMR), milked twice/day and artificially inseminated. The annual milk production per cow in the farm averaged 8500 kg energy-corrected milk. A professional hoof trimmer trimmed the cows' feet

routinely twice a year, in autumn and spring, using a transportable hydraulic trimming chute. Footbaths were not used on this dairy farm during the study. This study protocol was approved by the Committee of Animal Welfare and Ethics, Faculty of Veterinary Medicine, Mansoura University.

Treatment strategies. The routine practice for the herd was to identify all lame cows and to record the types of lesion and dates of treatment. According to lesion stage, cows with active lesions (M2) were enrolled for the study. Thirty Holstein-Frisian cows, presenting active DD lesions in their hind claws over the bulb of the heel, were allocated randomly to three treatment groups (10 each). The first group was subjected to topical treatment using 10g of antiseptic paste (3 g iodine + 2 g potassium iodine + 30 g zinc oxide)/100 g (Hokuto Pharmaceutical Company, Japan). The second group received topical treatment using 10g of antibiotic paste (250 mg/3 g of cefazolin, MSD Animal Health, Japan). The third group was treated topically with 10g of an antiseptic-antibiotic combination paste. The clinicians and participants were unaware of the upcoming topical treatment. All the claws affected with DD were subjected to mechanical debridement and cleansing before the topical treatment. Each cow was positioned on a transportable hydraulic hoof trim chute; all feet were rinsed with a water hose with a nozzle until the DD lesions were exposed for visual inspection. If the presence of manure prevented observation of lesions, this was removed with a towel. The surgical debridement involved the removal of all necrotic tissues, granulomas, and any remaining compromised tissues. The volumes of paste were similar and were sufficient to cover the lesion. The topical paste was applied immediately after claw debridement to a 10 × 10 cm cotton gauze and held in place using a light hoof elastic bandage (Vetrap Bandaging Tape, 3M Products) for 72 hours. After topical treatment, all the cows were kept on a dry surface for 30 min before returning to the stall. The treatment was repeated one more time during the 1st week. The hooves were considered to be cured once a complete epithelial layer had been established on the lesion (M0) and they were not painful upon palpation. Cows with signs of pain or an active lesion 28 days post-treatment were considered treatment failures. The clinical presentation of DD healing progress was recorded weekly and for 28 days after treatment using a standard protocol of a scoring system and a digital camera. During all observation periods, all feet lesions were washed and clinically scored for subjective evaluation afterwards.

Case definition scores. DD lesions were evaluated visually and by digital palpation. On the basis of clinical presentation, the lesion stage, depth and size were noted (Table 1). All clinical variables of DD were assessed and scored by one person, blinded to the treatment for the sake of consistency. The lesion stage was evaluated using a scoring system comprising five stages (M0-M4) according to DOPFER et al., 1997. While the lesion depth was assessed in the examination stanchion using a stainless steel probe, the lesion

size was measured at the widest part of the lesion using measuring tape. Resolution of DD lesions was defined as change from any lesion score to no lesion at the next observation.

Clinical index scores. Cows were clinically evaluated whilst walking and resting for subjective assessment of the clinical signs of DD. All these parameters were recorded and scored as clinical index scores by one person, to be evaluated and compared before treatment and throughout the duration of treatment (Table 2). Lameness was graded on a scale 0-3 (0 = no lameness; 3 = severe lameness). Pain was assessed by firm digital pressure with one thumb and shown by foot retraction (mild, score 1), exaggerated shaking of the foot (Moderate, score 2) and/or non-weight bearing (Severe, score 3). Discomfort was closely evaluated by daily recording alterations in normal activities, the appetite of the affected cow and changes to normal attitude and bouts of lying down.

Statistical analysis. Data were statistically analyzed using a statistical software program (GraphPad prism version 5.0, GraphPad software Inc., USA). Treatment outcomes were assessed first by evaluation of homogeneity in the groups of cows. The case definition and clinical index scores on the day of the first examination were compared between the groups by the Kruskal-Wallis nonparametric ANOVA test. Furthermore, two-way ANOVA was used to evaluate the effect of both time and treatment. Where the result was significant, differences between treatment groups at different time points were evaluated by the Kruskal-Wallis nonparametric ANOVA test. To assess the differences in the recovery rates between treatment groups, the chi-square test was used. Differences between median and range at $P<0.05$ were considered significant.

Results

Case definition score findings. The case definition scores of DD lesions treated with the topical paste showed a significant decrease in the lesion depth and size in the antiseptic-antibiotic group when compared with the antiseptic and antibiotic groups, especially at 21 ($P<0.05$) and 28 days ($P<0.05$) post-treatment (Fig. 1). The median and range for the lesion depth and size in the three groups are presented in Tables 3 and 4.

Clinical index score findings. The sum of clinical index scores showed a significant decrease ($P<0.05$) in the group treated with the antiseptic-antibiotic combination compared with the antiseptic and antibiotic groups at 14 days post-treatment. The median and range for the lameness, pain and discomfort of the treated groups are presented in tables 5-7.

Treatment outcomes. By the 28th day post-treatment, the statistical analyses revealed a significant increase ($P<0.05$) in the recovery rate over the time of treatment and between groups especially in cows treated with antiseptic-antibiotic paste (9/10) in comparison to the antiseptic (4/10) and antibiotic (5/10) treatment groups, as presented in Table 8.

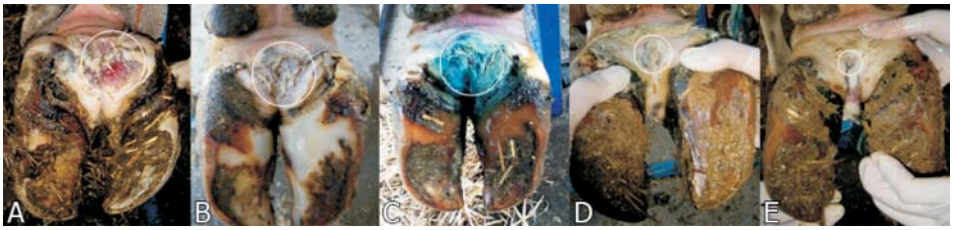

Fig. 1. Clinical appearance of a DD lesion on the hind claw of a Holstein-Friesian cow, pre-and post-treatment. A. On day 0 pre-treatment (after debridement); a Large size (>2 cm) active DD (M2) lesion. B. Lesion dryness by the 7th day post-treatment. C. On the 14th day of local therapy: lesion healing with a brown scab. D. Dyskeratosis with significant decrease in lesion depth and size at 21 days post-treatment. E. Complete resolution of the DD lesion by day 28 post-treatment.

Table 1. The case definition scores for subjective assessment of lesion parameters in 30 Holstein-Frisian cows with digital dermatitis.

Case definition index	Score and description	
Lesion Stage	0 ≡ No lesion (M0)	1 ≡ An early stage lesion (M1)
	2 ≡ The classical ulcerative stage (M2)	3 ≡ The healing stage (M3)
	4 ≡ The chronic stage (M4)	
Depth	0 ≡ No lesion	1 ≡ Shallow (< 2 mm);
	2 ≡ Proliferative (< 5 mm)	3 ≡ Deep (< 10 mm)
Size	0 ≡ No lesion	1 ≡ Small (< 1 cm)
	2 ≡ Medium (1-2 cm)	3 ≡ Large (> 2 cm)

≡ Means equal

Table 2. The clinical index scores for subjective assessment of clinical parameters in 30 Holstein-Frisian cows with digital dermatitis.

Clinical index	Score and description			
Lameness	0 ≡ Normal, cow stands and walks with a level back			
	1 ≡ Mildly lame, cow develops an arched back to stand and walk			
	2 ≡ Moderately lame, crched back is evident short strided gait			
	3 ≡ Severely lame, inability to bear weight on one or more feet			
Pain	0 ≡ Absent	1 ≡ Mild	2 ≡ Moderate	3 ≡ Severe
Discomfort	0 ≡ Comfort	1 ≡ Discomfort		

Table 3. Effect of topical treatment with antiseptic and/or antibiotic paste on lesion depth scores (median and range)

	Pre-treatment	Time post-treatment (days)			
Group	0	7	14	21	28
G1 (10 cows)	2 (1-3)	2 (1-3)	2 (1-2)	1 (0-2)[b]	1 (0-1)[b]
G2 (10 cows)	2 (1-3)	2 (1-3)	1 (1-3)	1 (0-3)[b]	1 (0-2)[b]
G3 (10 cows)	2 (1-3)	1 (1-3)	1 (0-3)	0 (0-2)[a]	0 (0-1)[a]

[a, b, c]: Medians and ranges with different superscript letters at the same column are significantly different at P<0.05. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste

Table 4. Effect of topical treatment with antiseptic and/or antibiotic paste on lesion size scores (median and range)

	Pre-treatment	Time post-treatment (days)			
Group	0	7	14	21	28
G1 (10 cows)	2 (0-3)	2 (1-3)	2 (1-2)	1 (0-3)[b]	1 (0-4)[b]
G2 (10 cows)	2 (1-3)	2 (1-2)	1 (0-2)	1 (0-2)[b]	1 (0-2)[b]
G3 (10 cows)	2.5 (1-3)	1.5 (0-3)	0.5 (0-2)	0 (0-2)[a]	0 (0-1)[a]

[a, b, c]: Medians and ranges with different superscript letters at the same column are significantly different at P<0.05. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste

Table 5. Effect of topical treatment with antiseptic and/or antibiotic paste on lameness scores (median and range)

	Pre-treatment	Time post-treatment (days)			
Group	0	7	14	21	28
G1 (10 cows)	2 (1-4)	2 (1-3)	2 (0-2)[b]	1 (0-2)[b]	1 (0-2)[b]
G2 (10 cows)	2 (2-4)	2 (1-3)	2 (1-3)[b]	1 (1-2)[b]	1 (0-2)[b]
G3 (10 cows)	2.5 (2-4)	1.5 (1-3)	1 (0-2)[a]	0 (0-1)[a]	0 (0-1)[a]

[a, b, c]: Medians and ranges with different superscript letters at the same column are significantly different at P<0.05. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste.

Table 6. Effect of topical treatment with antiseptic and/or antibiotic paste on pain scores (median and range)

	Pre-treatment	Time post-treatment (days)			
Group	0	7	14	21	28
G1 (10 cows)	3 (1-3)	2 (1-3)	1.5 (0-2)^{b}	1 (0-2)^{b}	1 (0-2)^{b}
G2 (10 cows)	2.5 (2-3)	2 (1-3)	2 (1-2)^{b}	1 (1-2)^{b}	1 (0-2)^{b}
G3 (10 cows)	2 (1-3)	1 (0-2)	0 (0-2)^{a}	0 (0-1)^{a}	0 (0-1)^{a}

$^{a, b, c}$: Medians and ranges with different superscript letters at the same column are significantly different at $P<0.05$. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste

Table 7. Effect of topical treatment with antiseptic and/or antibiotic paste on discomfort scores (median and range)

	Pre-treatment	Time post-treatment (days)			
Group	0	7	14	21	28
G1 (10 cows)	1 (1-1)	1 (1-1)	1 (0-1)^{b}	1 (0-1)^{b}	1 (0-1)^{b}
G2 (10 cows)	1 (1-1)	1 (1-1)	1 (0-1)^{b}	1 (0-1)^{b}	1 (0-1)^{b}
G3 (10 cows)	1 (1-1)	0.5 (0-1)	0 (0-1)^{a}	0 (0-1)^{a}	0 (0-1)^{a}

$^{a, b, c}$: Medians and ranges with different superscript letters at the same column are significantly different at $P<0.05$. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste

Table 8. Treatment outcomes of DD in 30 Holstein-Friesian dairy cows after topical treatment with antiseptic and/or antibiotic paste

	Recovery rates/days				
Group	0	7	14	21	28
G1 (n = 10)	0/10	0/10	1/10^{b}	2/10^{b}	410^{b}
G2 (n = 10)	0/10	0/10	1/10^{b}	3/10^{b}	5/10^{b}
G3 (n = 10)	0/10	1/10	4/10^{a}	8/10^{a}	9/10^{a}

Variables with different superscript letters at the same column are significantly different at $P<0.05$. G1: Animal group treated with antiseptic paste; G2: Animal group treated with antibiotic paste; G3: Animal group treated with antiseptic-antibiotic paste

Discussion

The individual use of antiseptic or antibiotic alone was not enough to treat DD lesions in dairy cows, despite the benefits inferred from the mechanical debridement of the DD lesion (CUTLER et al., 2013; GROENEVELT et al., 2014). Therefore, in the present study we assumed that the concomitant use of the antiseptic paste, with a reduced amount of a broad spectrum antibiotic such as cefazolin, would increase the potency of the antiseptic and minimize the amount of antibiotic residues.

The present results indicate that the antiseptic-antibiotic combination displayed better and more consistent results for treatment of DD within 14 days post-treatment, which was manifested by a significantly improved clinical index and case definition scores. The effect of the antiseptic-antibiotic paste for the treatment of DD could be attributed to the antiseptic and antimicrobial effect of the paste components. These small quantities of cefazolin and iodine, potassium iodine and zinc oxide salts are known for their antiseptic activity, bactericidal properties, and their ability to penetrate and protect the keratinized claw. Moreover, the paste is ecologically acceptable, economic and easily applied without a special instrument. These findings are in agreement with previous reports (STEVANCEVIC et al., 2009; HOLZHAUER et al., 2011; TOHOLJ et al., 2012).

Case definition evaluation for DD treatment with topical paste in dairy cows provides the opportunity to accurately evaluate different features of DD repair (RELUN et al., 2011). In the present study, based on the lesion depth and size scores, the proportion of lesion depth and size were significantly reduced by day 21 post-treatment in the antiseptic-antibiotic group compared to the cows treated with antiseptic and antibiotic pastes.

The anatomic location and the stage of DD lesions in dairy cows have an influence on the efficacy of the topical treatment (SHEARER and HERNANDEZ, 2000; RELUN et al., 2013). In this study, all the DD lesions were active and located in the hind claws, with typical localization on the planter skin over the bulb of the heel. This could be attributed to the closer contact of the heel bulbs, which are more prone to being continually moist, which favors the development of DD, especially M2 lesions (READ and WALKER, 1998). Also, the lesion site over the bulb of the heel keeps the medicament directly in contact with the active lesion and allows the lesion to dry out and begin healing within 3 weeks.

The clinical index scores provide a useful indicator of the locomotion state, comfort condition and vitality of the affected cows, pre- and post-treatment. Clinical recovery after successful topical treatment was represented by improved clinical index scores of all parameters. Our study was based on examination of affected dairy cows with DD for evaluation of lameness, pain and discomfort at each point in time, through fixed clinical index scores. In this study, cows treated with the antiseptic-antibiotic paste showed significant improvement in the clinical index scores when compared with groups treated with antiseptic or antibiotic paste in the second week of treatment.

There are varying degrees of lameness in cows suffering from active DD. In previous studies, cows with DD lesions showed moderate or slight lameness, but in other cows no lameness on walking could be observed (REFAAI et al., 2013; GROENEVELT et al., 2014). In the present study, comparable results were assessed, on day 0, when all cows with active DD lesions showed mild to severe degrees of lameness, which significantly reduced over the treatment period, especially in the cows treated with antiseptic-antibiotic paste.

The score system used for pain evaluation in this study seemed to serve well for proving the efficacy of any topical therapy. On day 0 before treatment all included cows were suffering mild to severe pain. By the day 14th post-treatment, the pain was significantly relieved in cows treated with antiseptic-antibiotic paste compared with cows treated with antiseptic and antibiotic pastes.

Depending on the intensity of DD, irritability and great sensitivity of the involved area may cause pain and discomfort to the animals, manifested by variant degrees of lameness and preference for lying down (ETTEMA and ØSTERGAARD, 2006). In this study, all affected cows showed increased discomfort scores on day 0 which significantly reduced following treatment, especially in cows treated with the antiseptic-antibiotic paste. This could be attributed to the nature of the M2 lesions, which are circumscribed areas of ulcerative, erosive dermatitis. Also, involvement of the dermis makes the lesions painful, and affected animals are lame and show discomfort.

Claw debridement and trimming before application of the topical paste is an essential step, advocated as an adjunct for all types of treatment used in this study. Mechanical debridement of the affected feet had some curative effect on DD by removal of all the visible dirt and exudate from the affected skin area, exposing lesions to air and thus desiccation. These findings are in accordance with MANSKE et al. (2002) and GROENEVELT et al. (2014).

An important aspect of any topical treatment is to keep the cows on a dry surface after local therapy before returning to the stall (CUTLER et al., 2013). In this way, a light hoof bandage was applied to cover the topical paste used in this study, and to ensure prolonged exposure of the affected tissue to the topical paste, to develop its antimicrobial effect.

The majority of DD lesion resolution occurs within 4 weeks of the disease (BERRY et al., 2008). In the present study, the rate of lesion resolution was evaluated through the weekly observation of the treated claws. The resolution of all DD lesions was observed within 14-21 days, especially in the cows treated with antiseptic-antibiotic paste. These present findings coincide with BERRY et al. (2008). In contrast, NIELSEN et al. (2009) reported that resolution of topically treated DD lesions in approximately 40 days.

The collaboration system between the clinical index scores and case definition scores used in this study provides a simple tool for subjective assessment of the effectiveness of antiseptic-antibiotic paste in treating DD in dairy cows. These findings were in accordance with BERRY et al. (2010) and RELUN et al. (2011).

The design of the DD scoring system used in this study allows us to assess the effectiveness of treatment trials in terms of the time needed in proportion to recovered DD claws. In most studies on topical treatments of DD, cure rates at day 30 of around 60-70 % have been found (BERRY et al., 2010 and 2012; CUTLER et al., 2013). The present study

indicated that the recovery rate by the day 28th of treatment was 9/10 in the antiseptic-antibiotic paste group, 5/10 in the antibiotic paste group and 4/10 in the antiseptic group, which is better than antiseptic or antibiotic treatment trials in previous studies.

The limitation of the present study was the small number of treatment groups. Another limitation is that, for animal welfare reasons we did not use a negative control group in which the cows were left untreated. Therefore, these shortcomings should be considered in a further study.

Conclusion

In conclusion, the clinically synergistic effect of the topical antiseptic-antibiotic combination appears to be an effective alternative treatment for DD in dairy cows, increasing the potency of the antiseptic and minimizing the amount of antibiotic residues. Further studies need to be done on different proportions of the antiseptic-antibiotic combination, to discover the optimal constituents with the minimal amount of antibiotic.

Conflict of interest statement

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Received: 29 April 2015
Accepted: 10 December 2015

EL-SHAFAEY, E., Y. ELSEADY, S. EL-KHODERY: Usporedno istraživanje terapijskog učinka antiseptično-antibiotske paste za lokalno liječenje digitalnog dermatitisa u mliječnih krava. Vet. arhiv 86, 197-208, 2016.

SAŽETAK

Istraživanje je provedeno radi procjene učinkovitosti istodobne primjene antiseptične paste sa smanjenom količinom antibiotika širokog spektra kao alternative za lokalno liječenje digitalnog dermatitisa (DD) u mliječnih krava. Trideset mliječnih krava holštajnsko-frizijske pasmine s aktivnim DD nasumce je bilo izabrano iz skupine od 93 oboljelih. Krave su bile nasumce raspoređene u tri pokusne skupine po 10 životinja. Jedna skupina bila je liječena antiseptičnom pastom (3 g joda, 2 g kalijeva jodida i 30 g cinkova oksida/100 g), druga antibiotskom pastom (250 mg/3 gm cefazolina), a treća antiseptično-antibiotskom pastom. Sve su životinje bile s laganim zavojem tijekom 72 sata. Klinički bodovni indeks i rezultati za pojedini slučaj bili su procijenjeni 0-tog dana, tj. prije liječenja te 7., 14., 21. i 28. dana nakon liječenja. Skupina liječena antiseptično-antibiotskom pastom ocijenjena je sa značajno manjim brojem bodova 21. i 28. dana nakon liječenja s obzirom na dubinu ($P<0,05$) i veličinu ($P<0,05$) lezija u usporedbi sa skupinama koje su bile liječene antiseptičnom ili antibiotskom pastom. Zbroj kliničkog bodovnog indeksa bio je značajno smanjen ($P<0,05$) u skupini liječenoj antiseptično-antibiotskom pastom u usporedbi s rezultatima za antiseptičnu ili antibiotsku skupinu 14. dana nakon liječenja. 28. dana broj oporavljenih u skupini liječenih antiseptično-antibiotskom pastom iznosio je 9/10, u skupini liječenih antibiotskom pastom bio je 5/10, a u skupini liječenih antiseptičnom pastom broj izliječenih bio je 4/10. Zaključno se može reći da rezultati upućuju na sinergistički učinak lokalno primijenjenog antiseptično-antibiotskog pripravka što se može rabiti za liječenje DD u mliječnih krava i za smanjenje količine rezidua.

Ključne riječi: antiseptično-antibiotska pasta, klinički indeks, krave, digitalni dermatitis