

STUDY ON THE EFFECTIVENESS OF TOPICAL APPLICATION OF ANTISEPTICS IN THE THERAPY OF DIGITAL DERMATITIS IN DIARY CATTLE

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Digital dermatitis (DD) in cattle is an infectious skin disease of the plantar side of the interdigital space. The causative agents are bacteria from the genus Troponema. Pathomorphologically DD is defined as a combination of ulcerative and proliferative changes which reach down to the dermal papillae and stick out from the ulcerous surface. In order for DD to develop, beside the infective agent, predisposing factors such as wet and unclean floors are required. Treatment of this disorder is possible with the application of antibiotics and/or antiseptic solutions. The aim of this study was to investigate the effectiveness of topical application of antiseptics in the therapy of DD lesions in affected dairy cattle. The applied antiseptic solutions were: copper sulfate (8%), zinc sulfate (8%), formalin (8%), and peracetic acid (3%). The treatment was carried out daily during the first seven days, thereon every second day for a total of 30 days. Chlortetracycline was used as the positive control and saline solution was applied as the negative control. By estimation of the presence, size and painfulness of the DD lesions we have established that zinc sulfate had the best curative effect when compared to other antiseptics.

Key words: claw disorders, digital dermatitis, antiseptics

INTRODUCTION

Since Cheli and Montellaro (1974) have described digital dermatitis (DD), it has become one of the most common infective diseases in cattle. The changes of the skin are localized on the plantar side of the interdigital space. Lesions show features typical for ulcerations, most often of a round or oval shape, red in color and very painful on touch. Pathomorphological aspects include a combination of ulcerative and proliferative changes which reach all the way down to the dermal papillae. Ulcerations are present in the form of circumscribed changes with hyperplasia of the epidermis and parakeratosis and hyperkeratosis on the margins of the lesions (Berry *et al.*, 2004). The disease has a multicausal etiology. Besides microorganisms, unhygienic housing conditions are a predisposing factor for DD (Somers *et al.*, 2005). By microscopic examination of the lesions a wide spectrum of bacteria can be identified, which indicates that DD is caused by a polymicrobial population (Edwards *et al.*, 2003). The superficial debris which

coats the ulcer contains numerous gram negative bacterial species (Choi *et al.*, 1997; Moter, 1998) whose etiological role in the development of DD is still unclear.

Bacteria from the genus *Troponema* are often found in the lesions characteristic for DD. They invade the deep skin layers, which incriminates them as the main causative agent. From within the lesions most commonly are identified: *Troponema denticola*, *Troponema brenaborense*, *Troponema phagedenis* and *Troponema vincenti/medium* (Trott *et al.*, 2003). It has been established that these bacteria are phylogenetically close to the same bacteria which cause gingivitis in man (Andrew *et al.*, 2003; Stamm *et al.*, 2006). However, some isolates are quite different from all others (Schrank, 1999). In addition to these bacteria, in the deeper layers of the skin *Guggenheimella bovis* (Sebastian *et al.*, 2008) has been recently identified. Due to the possibility of wide spreading within the herd, digital dermatitis is more often found in cows which are kept in a free stall system (Laven, 2004). The way in which the disease is spread is still unclear. However, it is known that for an artificial infection other predisposing factors are needed (Berry, 2004). Studies on artificial infections have proven that bacteria (anaerobic spirochetes) penetrate the skin if this is softened by excessive moisture (Read *et al.*, 1996). In the treatment of DD antibiotics and/or antiseptics are used. The treatment can be individual (topical application) or in groups (disinfecting barriers). Digital dermatitis is important for the economic effect of the production, as it results in decreased milk yield, poor reproductive performance, and early rejection from the production process and high costs of treatment procedures (Wells *et al.*, 1999).

MATERIAL AND METHODS

A total of 183 Holstein-Friesian cows, allotted to 4 experimental groups, were included in the trial. All the animals were housed in a free stall system. The average age of the cows was about 4 years (3.84 ± 1.26). All cows were in mid lactation (171 ± 35 days) (Table 1).

Table 1. Dairy cows included in the trial

Group	Group distribution of dairy cows			Number of dairy cows (n)
	Farm A	Farm B	Farm C	
E1	11	9	9	29
E2	10	9	13	32
E3	9	14	12	35
E4	12	8	7	27
K1	10	13	8	31
K2	9	5	15	29
Total	61	58	64	183
Age ($X \pm SD$)*	3.45 ± 1.08	4.06 ± 1.32	4 ± 1.38	3.84 ± 1.26
Days of lactation ($X \pm SD$)*	173 ± 32	185 ± 38	156 ± 26	171 ± 35

*X – mean value; SD – standard deviation

All 4 experimental groups were treated with antiseptics, thus avoiding the effect of the environment on experimental results.

Housing and maintenance

The cows were housed on 3 separate farms with a free stall system. The bedding was 180 x 120 cm and slightly elevated from the corridor area. The bedding and the corridors were cement built and covered with clean wheat straw on a daily basis. Removal of manure was carried out with the aid of a tractor with a built-in shovel. Feeding was at meal times and water was *ad libitum*. Forage consisted of corn and alfalfa silage, and alfalfa hay. The concentrated part of the meal consisted of a mixture of grains, vegetable oil industry by-products and vitamin-mineral supplements. Dairy cows were milked twice a day. Claw corrections were performed twice a year, while disinfection of the feet was inexistent.

Treatments

The treatment consisted of mechanical removal of the debris with a cotton swab dipped into sterile saline solution (Hemofarm, Vršac), followed by a topical application of a respective antiseptic solution. The antiseptic solution was sprayed on with an aerosol pump (Bonela, Odžaci). Group E1 was treated with 8% copper sulfate solution (Župa, Kruševac); group E2 with 8% zinc sulfate (Župa, Kruševac); E3 with 8% formalin solution (Formaldehyd, Centrochem) and Group E4 was treated with a 3% peracetic acid solution (Germiper®, NPK inžinjering, Belgrade). After application of the antiseptic solution the affected area was covered with a neutral Vaseline ointment. The treatment was repeated on a daily basis during a one week period and thereof every second day up to the 30th day from the start of the trial. The procedure was carried out after morning milking. The management of the negative control group (K1) consisted of only cleansing with saline and covering with neutral Vaseline ointment. The positive control (K2) was treated with chlortetracycline spray (Aueromycin, Fort Dodge, Spain) on a daily basis for a weekly period. Clinical examination of the present lesions was performed on the 15th and 30th day. The size, shape and degree of pain were estimated during the examination. The degree of pain was estimated by touching the lesion with a swab. The lesion score was: 0 = absence of lesions; 1 = concave or planar lesions, weak to moderate pain, smaller than 2 cm in diameter; 2 = planar lesions with present granulation tissue, painful, larger than 2 cm in diameter; 3 = large (chronic) lesions that are prominent over the surface of the surrounding skin, with or without papillomatous skin proliferates (Figure 1). This method is useful for the evaluation of the efficacy of DD treatment (Britt *et al.*, 1999). Comparison of the results and evaluation of the statistical significance level was done by analysis of variance.

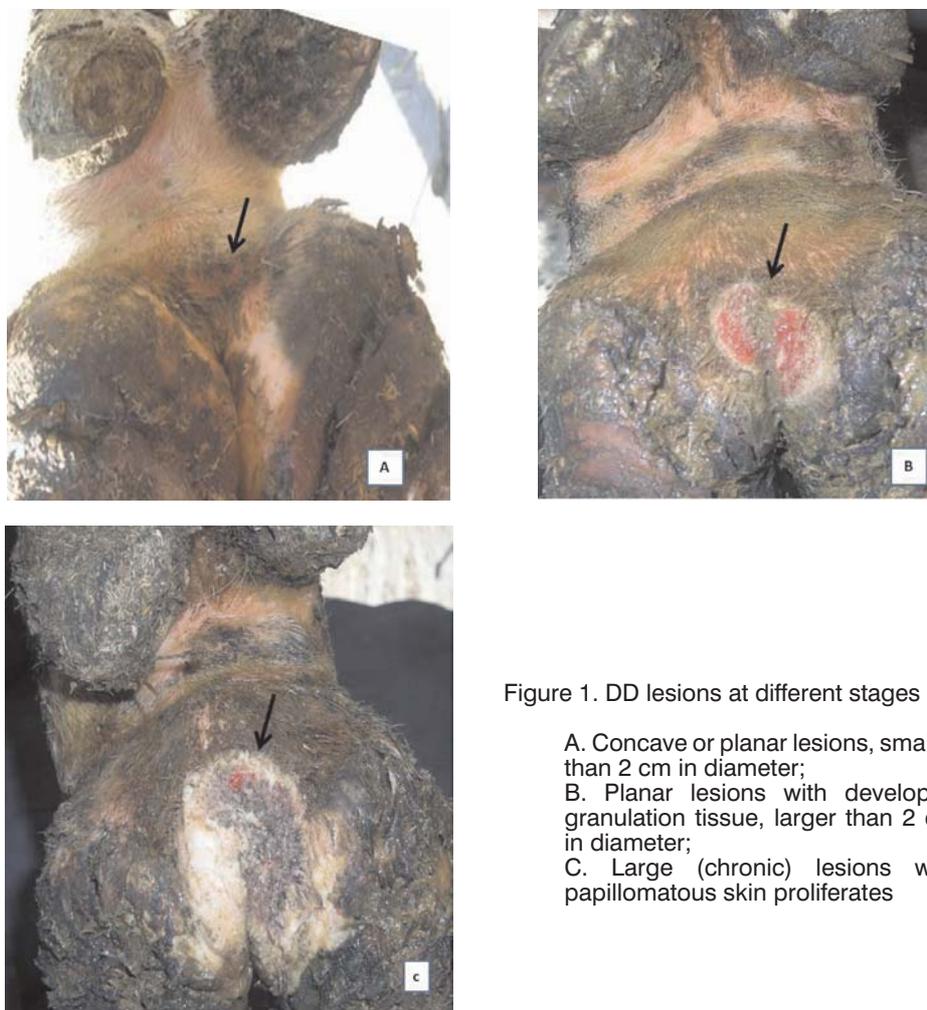


Figure 1. DD lesions at different stages

- A. Concave or planar lesions, smaller than 2 cm in diameter;
- B. Planar lesions with developed granulation tissue, larger than 2 cm in diameter;
- C. Large (chronic) lesions with papillomatous skin proliferates

RESULTS AND DISCUSSION

Estimation of the size and pain intensity of the lesions was done the first, 15th and 30th day after the morning milking session. In Table 2 are given the mean values for DD dermatitis obtained during the above period and described at clinical examination.

Table 2. Estimation of the effectiveness of antiseptics

Group	Treatment	Lesion score (X±SD)*		
		First day A	15th day B	30th day C
E1	Copper sulfate	2.10 ± 0.93	1.62 ± 0.89	0.91 ± 0.84
E2	Zinc sulfate	1.95 ± 0.95	1.15 ± 0.76	0.45 ± 0.56
E3	Formalin	2.01 ± 1.01	1.54 ± 0.89	1.11 ± 0.69
E4	Peracetic acid	2.14 ± 1.01	1.81 ± 0.99	1.29 ± 0.76
K1	Chlortetracycline	2.19 ± 0.90	0.83 ± 0.65	0.33 ± 0.47
K2	Saline	1.96 ± 0.93	2.30 ± 0.79	2.25 ± 0.78

*X – mean value, SD – standard deviation

On the first day of the treatment pain sensitivity was estimated with the aid of a swab stick. By testing the level of significance between the numerical values of the lesions no statistical significance was determined between the treated and control groups (Table 3).

Table 3. Variance analysis between groups on the first, 15th and 30th day of treatment

Day of clinical examination		Degrees of freedom	Mean squares	F value	P value
1.	effect	5	0.60	0.70ns	0.62
	error	360	0.86		
15.	effect	5	17.04	24.38**	3.7 E-21
	error	360	0.69		
30.	effect	5	28.7	59.46**	0
	error	360	0.48		

*p<0.05; ** p<0.01; ns p>0.05

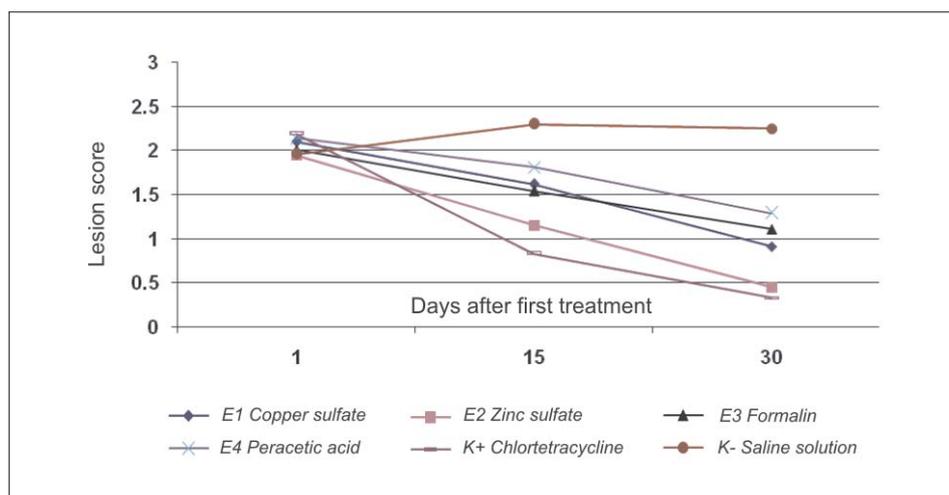
On the 15th day after the first treatment all lesions were clinically evaluated for presence, size and pain status of the DD lesions. Analysis of variance has shown that there is a statistically significant difference in the evaluation score between groups (Table 3). Individual tests (Table 4) have shown that there are significant differences in the degree of effectiveness between the tested antiseptics. Zinc sulfate has shown the highest efficacy in the treatment of DD lesions (1.95 on the first day and 1.15 on the 15th day). This effect was statistically significant when compared to the other studied antiseptics (copper sulfate, formalin and peracetic acid). At the same time, the best therapeutic effect was achieved in the positive control (K1) where the lesions were treated with the antibiotic chlortetracycline. Within the negative control group (K2) of diary cattle a progression in the score of the lesions was noted.

Table 4. Statistical significance of the differences of the effects of the applied antiseptics for the treatment of DD after 15 days of therapy

	E2	E3	E4	K1	K2
E1	≤ 0.01**	≥ 0.05 ns	≥ 0.05ns	≤ 0.01**	≤ 0.01**
E2		≤ 0.01**	≤ 0.01**	≤ 0.01**	≤ 0.01**
E3			≥ 0.05ns	≤ 0.01**	≤ 0.01**
E4				≤ 0.01**	≤ 0.01**
K1					≤ 0.01**

*p<0.05; **p<0.01; ns p≥0.05

On the 30th day of treatment all DD lesions were, once again, clinically evaluated for presence, size and pain status. At this time, all tested antiseptics have resulted in a significant improvement of the lesions (Graph 1). Of all the tested antiseptics zinc sulfate has shown the highest effectiveness in the treatment of DD, which has been proven by statistical means. The effect of zinc sulfate was after 30 days of application close to the effect of chlortetracycline used as the positive control. The difference in the effect of copper sulfate and formalin was not statistically significant (Table 5). Peracetic acid had the weakest effect compared to all the other tested antiseptics.



Graph 1. Score of DD lesions on the first, 15th and 30th day of treatment

Spontaneous DD wound healing rarely happens. Surgical removal of the proliferative lesions results in epithelisation and healing if the surgical field is maintained clean, which is very difficult to obtain (Grenough *et al.*, 1997). As DD is a disease caused by an infective agent, therapy consists mainly in the application

of antibiotics and/or antiseptics. Troponema is *in vivo* sensitive to a number of antibiotics. However, the positive therapeutic effect of their parenteral application is seldom seen (Blowey *et al.*, 1988). Due to poor efficacy, long withdrawal period and high costs parenteral application of antibiotics is not recommended for the treatment of DD. The most commonly used antibiotics are: tetracycline, erythromycin and lincosamine (Berry *et al.*, 1996). Application can be individual (topical application) or in groups as solutions are applied within the disinfecting barriers which are usually placed on the exit of the milking area.

Table 5. Statistical significance of the differences of the effects of the applied antiseptics for the treatment of DD after 30 days of therapy

	E2	E3	E4	K1	K2
E1	≤ 0.01**	≥ 0.05ns	≤ 0.01**	≤ 0.01**	≤ 0.01**
E2		≤ 0.01**	≤ 0.01**	≥ 0.05ns	≤ 0.01**
E3			≥ 0.05ns	≤ 0.01**	≤ 0.01**
E4				≤ 0.01**	≤ 0.01**
K1					≤ 0.01**

* $p \leq 0.05$; ** $p \leq 0.01$; ns $p \geq 0.05$

In this study chlortetracycline was very effective in the therapy of DD lesions. In the studied dairy cattle herds this antibiotic has never been used for DD therapy. However, authors have described a poor effect of chlortetracycline in stables in which this antibiotic has been added to the solution in the disinfecting barriers, thus indicating the development of bacterial resistance (Shearer *et al.*, 2000). Due to this in the USA the use of antibiotics in disinfecting barriers has been abolished. The effect of antiseptics is explained by non specific mechanisms which do not allow bacterial resistance to develop. The most commonly used antiseptics for treatment and prevention of DD are copper sulfate, formalin and zinc sulfate. The major setback for the use of carbon sulfate is that it represents a potential ecological hazard and it causes pain to the animal suffering from lesions of the acropodium (Bergsten *et al.*, 2003). In this study copper sulfate has shown a mediocre efficacy in the treatment of the lesions of the acropodium. This is in accordance to the previously published data (Nutter *et al.*, 1990; Rodriguez *et al.*, 1996). However, other studies have described a superior efficacy (Laven and Hunt, 2002). A relatively good effect of copper sulfate in our study can be explained by the fact that we have applied a higher concentration compared to previously published studies. An additional factor lays probably in the fact that we have covered the lesions with a neutral ointment which had a supplementary protective effect.

Zinc sulfate has a certain advantage as it is a bactericide, penetrates in the deeper layers of the tissue, protects the keratinous part of the claw, is less toxic and is ecologically acceptable (Radojčić *et al.*, 2005). In this study zinc sulfate has displayed a good effect in the treatment of DD lesions. On the 30th day of the trial the final effects of chlortetracycline and zinc sulfate did not differ. This is a

significant finding as it speaks out that that DD, as well as other claw soft tissue disorders, can be treated with antiseptics, without the use of antibiotics. Besides, zinc is an essential microelement which takes part in enzymatic reactions vital for keratin and collagen synthesis which is the building protein of the skin (Siciliano-Jones, 2008). Thus, zinc is a mandatory ingredient in vitamin-mineral supplements for dairy cows. Intake of adequate quantities of zinc results in improved morphological and functional characteristics of the bovine acropodium (Tomlinson *et al.*, 2004).

The effect of formalin is explained by coagulation necrosis protein denaturation. Formalin must be applied with up most care, as it is described as a carcinogenic substance (Laven and Hunt, 2002). In this study the effectiveness of formalin is close to the effectiveness of copper sulfate.

Peracetic acid represents a stabilized water solution with acetic acid and hydrogen peroxide. In this study peracetic acid has shown a poor effect compared to other treatments. This result is in accordance with the findings of Laven and Hunt (2002). The authors report a high efficiency of peracetic acid when applied as a special formulation (Kovex® foam system) with glycerin in the disinfecting barriers placed in the corridor leading to the milking area (Fiedler *et al.*, 2004). This mode of application ensures a longer lasting contact compared to the water solution. In our trial the application was carried out in such a way that the time of contact was short, which is probably the cause of the poor performance showed by this antiseptic solution compared to results published by other authors.

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**ISPITIVANJE EFIKASNOSTI TOPIKALNE PRIMENE ANTISEPTIKA U LEČENJU
DIGITALNOG DERMATITISA KRAVA**

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SADRŽAJ

Cilj ove studije je bio da se ispita efikasnost topikalne primene pojedinih antiseptika u terapiji digitalnog dermatitisa mlečnih krava. U terapiji su korišćeni sledeći antiseptici: bakar sulfat (8%), cink sulfat (8%), formalin (8%) i persirćetna kiselina (3%). Terapija je sprovedena svakodnevno tokom prve nedelje a zatim svakog drugog dana do isteka perioda od mesec dana. Hlortetraciklin je korišćen kao pozitivna a fiziološki rastvor kao negativna kontrola. Na osnovu procene prisustva, veličine i bolnosti lezija dokazano je cink sulfat ima najbolji terapeutski efekat u poređenju sa drugim primenjivanim antisepticima.