VETERINARY EVIDENCE online

A Randomised Non-inferiority Trial on the Effect of an Antibiotic or Non-antibiotic Topical Treatment Protocol for Digital Dermatitis in Dairy Cattle

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ABSTRACT

Objective:
Investigation of the therapeutic effect of a protocol using non-antibiotic Intra Epidine (IE) spray containing copper and zinc chelate on M2 digital dermatitis (DD) lesions compared to a treatment protocol using antibiotic chlortetracycline (CTC) spray for non-inferiority testing.
Background:
Digital dermatitis (DD) is an infection in dairy cattle which frequently results in lameness. A common individual treatment for DD in Europe is a topical administration of antibiotic CTC spray. Given the important objective to minimise antibiotic use in order to prevent selection for antibiotic resistance, there is a need for effective non-antibiotic treatments for common animal diseases.
Evidentiary value:
This clinical trial on commercial farms with registered products is valuable for all veterinary practitioners interested in the quantitative curative effects of treatments forDD.
Methods:
Professional hoof trimmers trimmed hind legs of 944 cows from nine dairy herds. All legs with DD M2 lesions were included and randomly assigned to a treatment protocol with IE spray or CTC spray according to the label instructions from the manufacturers. At the end of the study 231 individual legs, one per cow, in seven herds were eligible for analysis. Clinical improvement was defined as the transition of the clinical most relevant ulcerative M2 lesion to any other less severe lesion stage at 10 days after the start of the treatment.
Results:
The overall individual leg clinical improvement rate of IE spray (86.8%) was higher compared to CTC spray (47.9%). The herd adjusted odds ratio for clinical improvement was 8.2 (95% CI 4.2 – 15.7) for IE spray versus CTC spray with an estimated Relative Risk of 1.9.
Conclusion:
In conclusion, the IE treatment protocol was non-inferior but more effective than the CTC treatment protocol to clinically improve DD M2 lesions.

INTRODUCTION

Digital dermatitis (DD), also called Mortellaro's disease, is a superficial, painful, contagious inflammation of mainly the epidermis of the feet (Schroeder et al., 2003; Döpfer et al., 1997) that occurs worldwide (reviewed by Refaai et al., 2013) and frequently results in lameness. It affects not only the welfare of the animals but also gives rise to significant economic losses (Bruijnis et al., 2010; Cha et al., 2010; Enting et al., 1997). Typically, the skin adjacent to the interdigital space at the plantar side of the hind claws is affected and may cause animals to walk on the toes (Blowey, 1987; Read and Walker, 1998). The disease is observed in cattle of all ages.

A wet and dirty environment is an important risk factor for DD as pathogens can survive easily and foot hygiene is generally poor (Brizzi 1993; Somers et al., 2005a). Cows kept under drier circumstances therefore suffer less from DD (Brizzi, 1993). Other risk factors are floor type, access to pasture and hoof trimming interval (Holzhauer et al., 2006; Somers et al., 2005a; Somers et al., 2005b). In the Netherlands, about 21% of dairy cows were found to have one or more DD lesions, while within herd prevalence up to 83% were reported (Holzhauer et al., 2006).

There are five stages of DD, M0 to M4, described by Döpfer et al. (1997). Although recent investigations utiliSe a six-stage system for DD (Berry et al., 2012), the five-stage system by Döpfer et al. (1997) is generally applied in the Netherlands and therefore was used during the trial. An M0 stage relates to normal skin where no DD lesion is visible; an M1 relates to an early stage lesion of up to 2 centimetres; an M2 relates to the ulcerative,

'strawberry-like' lesion of more than 2 centimetres which is usually very painful; an M3 relates to a healing stage when the lesion is covered by a scab and an M4 relates to the chronic stage with a proliferative lesion (Döpfer et al., 1997). The aetiology of DD seems to be multifactorial, while spirochetes of the Treponema genus seem to be involved frequently (Döpfer et al., 1997; Stamm et al., 2002; Gomez et al., 2012; Rasmussen et al., 2012). Mumba et al. (1999) observed that the presence of spirochetes was relatively higher at M2 stage compared to any other stage. This might explain why most clinical signs are seen at this stage and also suggests that the M2 stage is the most infectious to other animals.

Digital dermatitis can be treated individually or at herd level. A herd level treatment may consist of a walkthrough footbath with zinc sulphate, copper sulphate, antibiotics or, in the Netherlands, a 4% formalin solution (Holzhauer et al., 2012; WVAB, 2016). A common individual treatment is the topical administration of chlortetracycline (CTC) spray, with the antibiotic chlortetracycline as its active compound (WVAB, 2016; CBG-MEB, 2013; Holzhauer et al., 2011). In several countries, the use of antibiotics in footbaths is not licensed and abundant use of antibiotics in farm animals is increasingly criticiSed for the potential selection of antibiotic resistance. Thus, obtaining effective preventive measures and treatments of DD without antibiotics is desirable. An individual topical treatment with a gel containing copper and zinc chelate was already shown to be effective (Holzhauer et al., 2011), but it was suggested that a spray would be more convenient to use than a gel.

The aim of this study was to investigate the therapeutic effect of Intra Epidine spray containing copper and zinc chelate (IE; Intracare, Veghel, The Netherlands) on M2 DD lesions compared to a treatment with CTC spray (CTC; Eurovet Animal Health, Bladel, The Netherlands) in the context of non-inferiority testing for market authorisation.

METHODS & MATERIALS

Selection of herds

Intracare (Veghel, The Netherlands) selected participating herds for the trial using several selection criteria. For practical and logistic reasons, we aimed to include herds of at least 100 cows with a DD prevalence of 20-25 percent in order to obtain a reasonable number of observations per farm. Furthermore, it was preferred to include herds that reflected the average situation in the Netherlands, with Holstein Friesian cows and freestalls. During the trial, it appeared that the strict use of these criteria did not result in the recruitment of sufficient affected legs. Therefore, it was accepted that not all herds fulfilled the requirements. They for example did not reach the minimum of 100 dairy cows or appeared to have a prevalence of DD of less than 20 percent. One herd had access to pasture during the study, whereas all other herds were housed indoors. Farmers were not allowed to use walk-through footbaths or other treatments in the last three weeks before and during the trial. All participating herds were located in the northern part of the Netherlands. The trial took place from October until December 2013, at the start of the indoor housed winter period.

Selection and treatment of cows and legs

Professional hoof trimmers trimmed the hind legs of the cows on day 0. Four observers were trained with photos and clinical cases. They collectively selected all hind legs with DD in the trimming chute and scored the lesions, using the scoring system described by Döpfer et al. (1997). They included only hind legs that were scored with ulcerative M2 lesions by all of them for this study as this lesion was regarded most relevant from a clinical point of view (Döpfer et al., 1997; Mumba et al., 1999). One leg was regarded as experimental unit. The

first hind leg with an M2 lesion in a herd was allocated in one of the treatment groups A or B by flipping a coin. The following hind legs with M2 lesions were alternately allocated to the two treatments. When both hind legs of a cow were affected, each was treated with a different product. All included legs were photographed, followed by the labelled used of two different products (treatment A and B). Treatment A consisted of an administration of IE spray, once for 3 seconds from 15 centimetres distance, on day 0. The observers covered the lesions, after drying for 30 seconds, with cotton wool, held in place by an elastic bandage (CoRip Flexible Cohesive Bandage GB11). The bandage was removed on day 3 and the legs were sprayed again with IE spray for 3 seconds on day 3 and day 7, but left uncovered. Treatment B consisted of a therapy with CTC spray according to the instructions of the manufacturers. Thus, the observers spraying the lesions two times for 3 seconds from 15 to 20 centimetres distance with a drying interval of 30 seconds on day 0. This treatment was repeated on day 1 and day 2. All repeated treatments were applied by the four observers as well, in groups of two. After consulting the ethical committee of Utrecht University, it appeared not necessary to obtain formal ethical approval as the trial was performed with registered active compounds approved to use on animals.

Scoring and blinding

The same four observers as day 0 scored and photographed all lesions during the treatment evaluation on day 10. Clinical improvement was defined as the transition of an ulcerative M2 lesion to any other lesion at day 10, either M0, M1, M3 or M4. All observers collectively checked all included legs for side effects at each treatment moment (day 1, 2, 3, 7, 10). Two independent trained and experienced observers, not actively involved in the data collection, checked inclusion and clinical improvement of legs afterwards, using the photographs. Blinding was not always achieved as the photographs occasionally showed a touch of green or blue from respectively IE spray and CTC spray. The colours of the products are well known to everybody working in this field and it was not possible to change the colours for the sake of this trial.

Sample size

Sample size was calculated for non-inferiority testing of 10 percent relative to the antibiotic product using nQuery (Cork, Ireland). The expected improvement rate of CTC spray, based on research of Holzhauer et al. (2011), was 60 percent. Thus, improvement rate of IE spray should be higher than 50 percent to be considered not inferior to CTC spray. The researchers assumed that healing of lesions within 10 days was not greatly affected by the fact that animals of both treatment groups were housed in the same herd. Therefore, clustering of cows within herds was neglected for the power calculation. Considering 95 percent confidence and 80 percent power this resulted in a sample size of 300 hind legs with M2 lesions for each group. After the first batch of seven farms at approximately half of the sample size, a planned interim analysis showed that non-inferiority was clearly present and therefore the trial was stopped.

Statistical methods

The data was analysed before unblinding of the treatments. One leg was randomly selected from cows with two affected legs. Risk differences were calculated for comparisons within individual herds. A logistic regression model with herd and treatment as fixed factors was used to estimate an adjusted odds ratio over all herds. With the herd adjusted results of the logistic regression, an estimated relative risk was calculated using the method described by Beaudeau and Fourichon (1998). Basically, the relative risk is recalculated based on the point estimate of the odds ratio from the multivariable logistic regression, in combination with the observed incidence rates in both treatment groups (not case-control design). For illustration purposes, calculations of the confidence intervals of the treatment difference were calculated using the square root of the sum of the variances of the two treatments (Schukken et al., 2013).

RESULTS

Descriptive statistics

During this study 285 legs in seven different herds were treated, of which 143 with IE spray and 142 with CTC spray. No adverse effect of either treatment was observed. One IE treated cow was found to have a swollen claw and was diagnosed with foot rot at day 1. The animal was treated by a veterinarian and excluded from the analysis. Another cow was excluded from the CTC group due to missing data. The resulting 283 treated legs were found in 231 cows, indicating that 52 animals had two affected legs. For the analysis, one leg of each of these pairs was randomly removed from the dataset, leaving 231 non-paired legs eligible for analysis. A flow diagram of leg inclusion is depicted in figure 1.

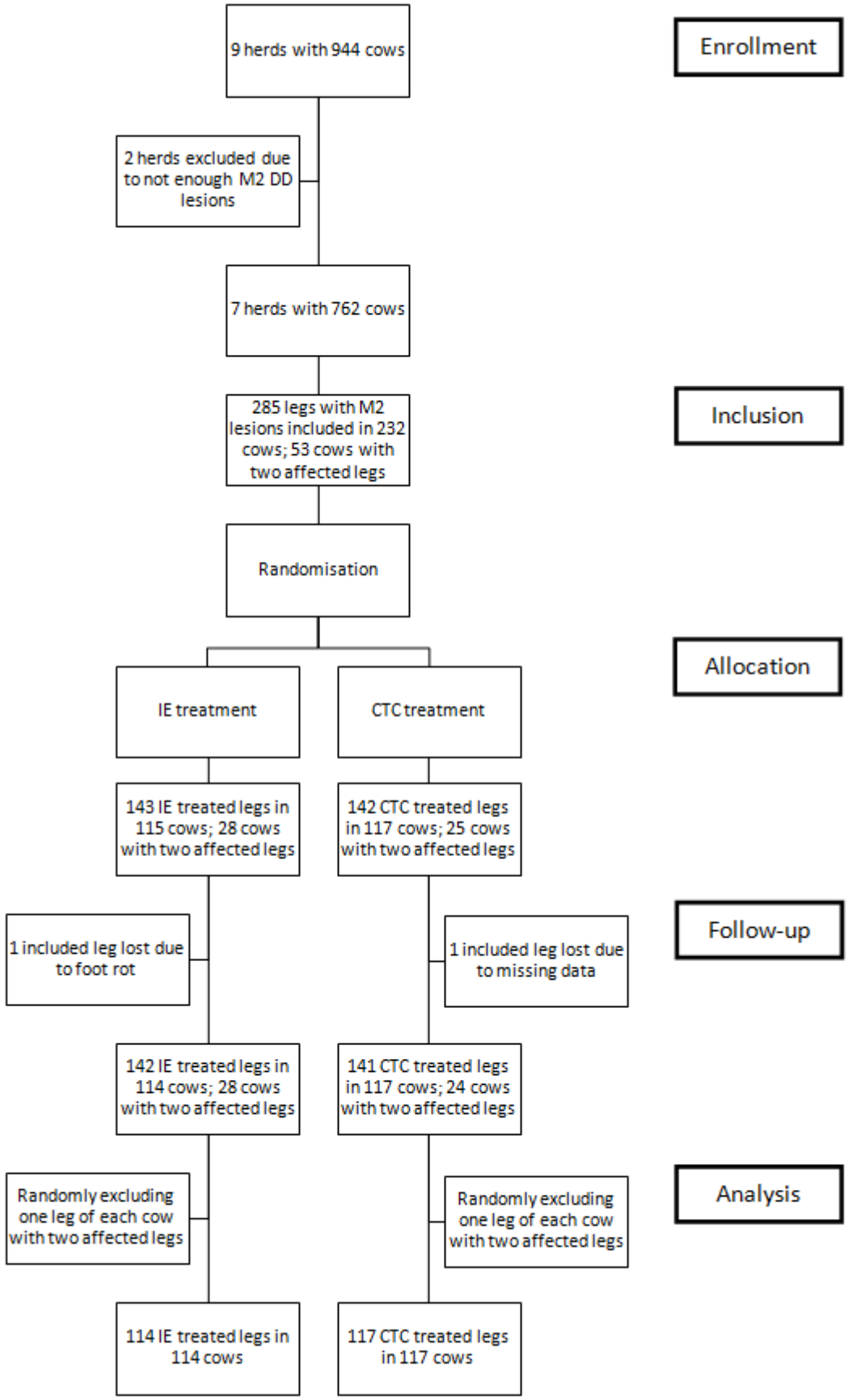

Figure 1: Flow chart of leg inclusion, according to REFLECT (2010).

Treatment outcome

The distribution of the scores on day 10 is depicted in figure 2A and 2B. After treatment with IE spray, most M2 lesions (71%) transitioned into M3. The percentage of M1 and M2 lesions was almost equal (respectively 14% and 13%). After treatment with CTC spray most lesions remained M2 (52%). The percentage of M1 lesions after treatment with CTC spray is a little higher compared to treatment with IE spray (19%). Only 1% and 3% for respectively IE spray and CTC spray transitioned to M0.

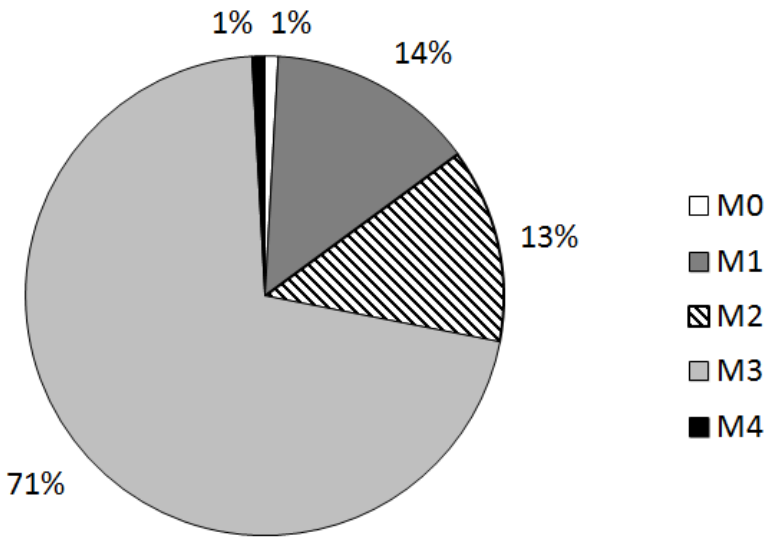

Figure 2A: Percentages of DD stages of 114 included legs in seven dairy herds, 10 days after first treatment of M2 lesions with IE spray.

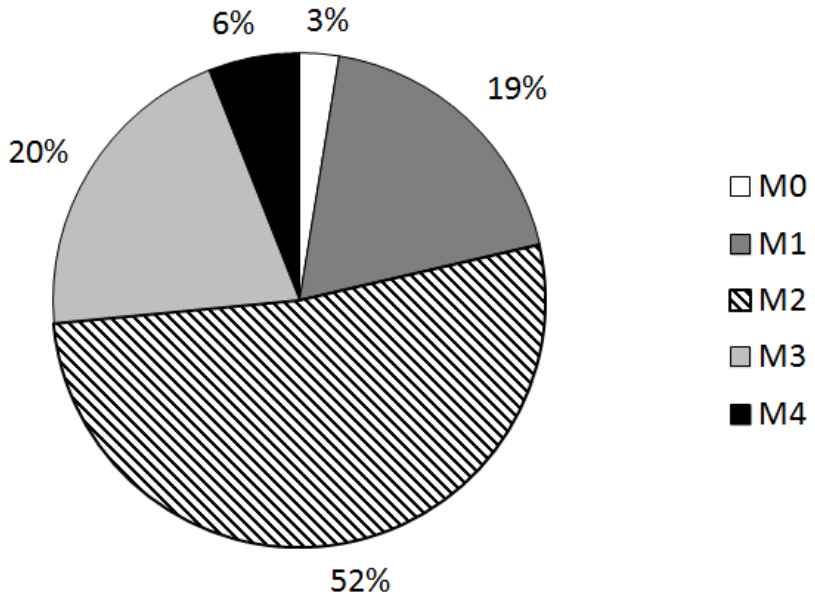

Figure 2B: Percentages of DD stages of 117 included legs in seven dairy herds, 10 days after first treatment of M2 lesions with CTC spray.

Clinical improvement rate

Table 1 shows the number of included legs and the number of clinically improved M2 lesions on day 10. The overall clinical improvement rate was 86.8% (herd range 61.5% - 100.0%) for IE treatment and 47.9% (herd range 17.7% - 85.2%) for the CTC spray. There was no interaction between herd and treatment.

Table 1

Number of included legs with an M2 lesion (one per cow) in each herd and their clinical improvement rate after 10 days for IE spray and CTC spray.

Herd	Number of legs	IE spray protocol		CTC spray protocol	
		Number improved / number treated (%)		Number improved / Number treated (%)	
1	17	10/10	(100.0)	6/7	(85.2)
2	30	8/13	(61.5)	3/17	(17.7)
3	24	10/12	(83.3)	7/12	(58.3)
4	69	28/32	(87.5)	13/37	(35.1)
5	22	8/9	(88.9)	5/13	(38.5)
6	16	8/9	(88.9)	4/7	(57.1)
7	53	27/29	(93.1)	18/24	(75.0)
Total	231	99/114	(86.8)	56/117	(47.9)

Risk Differences

Figure 3 illustrates the clinical improvement, which was numerically higher for IE spray compared to CTC spray and significantly higher in three herds (herd 2, 4, 5). The graph shows that IE spray is non-inferior and might be superior. This was confirmed in the logistic regression, in which clinical improvement was statistically different between treatments ($p<0.001$) and between herds ($p<0.001$). The odds ratio, adjusted for herd effects, for IE spray versus CTC spray was 8.2 (95% CI: 4.2 – 15.7). The estimated relative risk for clinical improvement derived from this adjusted odds ratio was 1.9 for IE spray versus CTC spray.

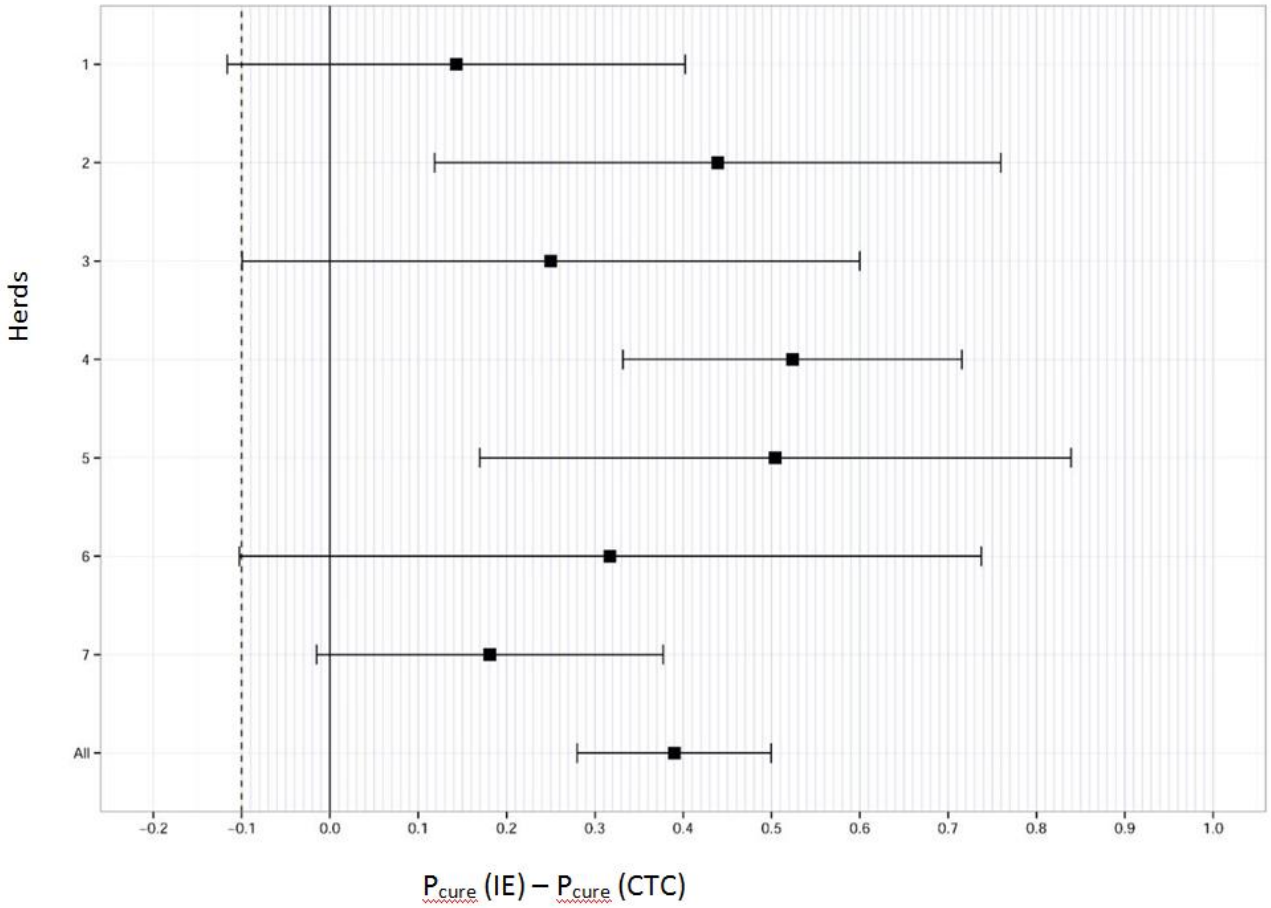

Figure 3: Overall and per herd estimated risk difference for clinical improvement of digital dermatitis M2 lesions. Black boxes and bars indicate the risk differences with their 95% confidence intervals. The shaded area right from the vertical interrupted line indicates the zone of non-inferiority for treatment with IE spray compared to CTC spray.

DISCUSSION

The overall clinical improvement rate of IE spray in the selected herds was 86.8% (range 61.5% - 100.0%). This is similar to the clinical improvement rate of an IE gel with similar active components 92% (range 86% - 94%) as found by Holzhauer et al. (2011). The overall clinical improvement rate of CTC spray varied greatly from 17.7% to 85.2% between farms. Improvement rates of 58% (Holzhauer et al., 2011), 68% (Berry et al., 2010) and 87% (Manske et al., 2002) were reported for either CTC or comparable oxytetracycline spray. This may be explained by differences in follow-up period between studies. Potential antibiotic resistance due to regular use of CTC spray on selected farms could play a role as well, as observed by Shearer et al. (2002).

Apart from differences in active components between treatments with IE and CTC spray, the application protocol differed as well, as legs were treated in compliance with manufacturer's instructions. Application of a bandage, as is done after treatment with IE spray, protects the lesion from dirt, prevents the medication from draining away and may have been a factor in the observed clinical improvement. Conversely, a bandage is likely to create a moist, anaerobic environment. As the spirochetes associated with DD are usually regarded as anaerobic, it could be speculated that using the bandage may also hamper clinical improvement (Holzhauer et al., 2006; Schroeder et al., 2003; Somers et al., 2005a). The interval between last application and evaluation

also differed for both treatments. The observed differences in clinical improvement may result from differences in active components, different application protocols, difference in time after last application, or a combination of these factors. These issues cannot be differentiated within the current study design and would need further investigation.
Clinical improvement of M2 lesions was defined as the primary outcome, as these severe ulcerative and highly painful lesions are related to clinical lameness. All transitions to other lesion categories indicate clinical improvement, which is certainly not the same as bacteriological cure. Thus, transition of a M2 lesion to M0, M1, M3 and M4 were weighed similar, although long term effects on herd level might be different. It was decided to use a short term study, in order to minimize effects of reinfections from the environment (Berry et al., 2010; Berry et al., 2012). This study period showed a difference in short term clinical improvement between the two topical treatments. Significant farm effects were observed, in particular a larger variation in short term clinical improvement for CTC spray and a better clinical improvement for the treatment protocol with IE spray.
It seems unlikely that topical treatment with either of the investigated drugs would result in elimination of DD within herds, because of persistent reservoirs of infection in the environment and untreated cows. Measures at herd level such as periodically hoof trimming, improvement of hygienic conditions and the use of footbaths may be more effective (Holzhauer et al., 2006; Holzhauer et al., 2012; Somers et al., 2005a). On the other hand, a reduction of the painful ulcerative M2 lesions in both treatment groups was observed, resulting in pain reduction and higher well-being for the affected animals.

CONFLICT OF INTEREST

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Gerwen Lammers is an employee of Intracare and contacted potential farmers and arranged that all cows were trimmed by professional hoof trimmers, and that included cows treated. They also checked the manuscript for mistakes.

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