THE INFLUENCE OF AN AEROSOL WITH ANTIBIOTICS ON THE WOUND HEALING FOLLOWING CAUTERY DISBUDDING OF PREWEANED DAIRY CALVES COMPARED WITH WOUND HEALING AFTER THE USE OF AN AEROSOL WITHOUT ANTIBIOTIC

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ABSTRACT

Disbudding dairy calves is still a common procedure, since it prevents horn-related trauma and allows for safe cattle handling. Calves endure increased pain sensitivity until the wounds have healed, which takes weeks. Thus, it is of interest to evaluate practical strategies to hasten healing after disbudding. Most veterinary practioners in the Netherlands use an aerosol spray to promote wound healing after disbudding. CTC-spray®, an aerosol spray that contains chlortetracycline, is probably most commonly used for this purpose. Since the use of antibiotics for several indications is frequently questioned and there is a lack of evidence for the use of antibiotics for this purpose, the goal of this double blinded field study was to evaluate wound healing following cautery disbudding using an aerosol with or without antibiotics. 255 dairy calves on 37 farms of 2 veterinary practices were disbudded using a standard cautery disbudding protocol. After disbudding, the two horn buds of each calf were treated with two different sprays. We alternately treated the left and right horn bud with an aerosol spray that contains chlortetracycline (CTC-spray®) and with a blue aerosol spray without antibiotics, called Keno™Fix. The primary outcomes were lesion score (LS) and wound diameter (WD) measured at 1 week and 4 weeks post disbudding. The LS was scored on a scale of 1 to 3, with LS = 1 representing normal healing with no scabs or discharge present, LS = 2 having scabs or raised crusts and LS = 3 showed moist or dried purulent discharge. The WD was measured in millimeters at the widest point of the inner edges of the wounds. Statistical analysis of the WD showed a significant difference at week 4 that was estimated at 0.35 mm in favor of CTC-spray®. This difference is too small to be of clinical importance. The odds to get a LS =2 or 3 was 2.5 times higher for Keno™Fix than for CTC-spray®. But, since this is not combined with a difference in wound diameter it is arguable whether the higher LS is really something that causes delayed healing or whether it is a normal stage of wound healing. The decision to use one of both sprays depends on the goals of the veterinary practice and the farmer, considering that we want to decrease the use of antibiotics, but the surface of the wound will be not as good looking, when we do.

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INTRODUCTION

It is known that there are frequently ranking interactions between cows in a herd. As the animals usually have horns, these interactions will result in trauma, particularly when the animals are kept in a confined area. Farmers want to prevent these interactions from resulting in horn-related trauma and bruising of carcasses at slaughter. Apart from the welfare issues for the animals and the related economic consequences, it is also less safe for farmers when handling horned instead of non-horned cattle (Stafford et al., 2010; Ramsay et al., 1976).

Horn growth can be prevented by genetic selection. The genetic selection for polled cattle is increasing, but is still only practiced in 2.5% of all inseminations in The Netherlands (Groen kennisnet, 2015). Disbudding procedures will therefore remain a standard aspect of dairy calf processing until polled sires are commonly used throughout the dairy farming industry. According to the Royal Dutch Association for Veterinarians (KNMvD), it is necessary that, until there is a solution in housing systems or until all cows are polled, farmers and veterinarians make sure that disbudding is performed with as little pain as possible.

In the Netherlands disbudding of calves is allowed, given that the disbudding is performed prior to the age of two months at the instruction of a veterinarian and with local analgesia (art. 4, paragraph n, Regulation for permitted acts). The general inspection service (AID) published that in the Netherlands in 2006 700.000 calves were dehorned by hot-iron disbudding (Houkema, 2010).

With the growing social concern and awareness for animal welfare, the disbudding of dairy calves has received increasing public attention. Therefore, a lot of research was performed on different methods of disbudding with different protocols for pain relief, measured by physiologic, behavioral and performance responses in calves (Stafford and Mellor, 2005). However, there are not many studies on wound healing, pain and performance of the calves during the wound healing process (Huebner et al., 2017).

It is known that there is increased pain sensitivity during 6 to 13 weeks until complete re-epithelialization of the wound has occurred (Adcock and Tucker, 2018). Another study reported increased sensitivity for at least 14 weeks (Casoni et al., 2019). Adcock and Tucker found that age at the time of disbudding did not affect latency to re-epithelialize or wound sensitivity. However, rump sensitivity was greater in calves disbudded at 3 versus 35 d of age on different moments within a window of two months after disbudding, which is in agreement with other studies showing increased pain sensitivity when painful procedures are performed at an early age (Adcock and Tucker, 2018). In Huebner et al., wound healing following cautery disbudding was evaluated after the appliance of aluminum-based aerosol bandage. The ALU-spray improved wound healing following cautery disbudding in pre-weaned dairy calves (Huebner et al., 2017).

There is a lot of knowledge about wound healing after burn injuries in humans. It appeared that burn wounds have more fundamental damage to tissues than nonburn trauma, which complicates the normal wound healing response (Rose and Chan, 2016). Successful wound healing occurs in a progression of phases typically classified as hemostasis, inflammation, proliferation, and maturation/remodeling (Shakespeare, 2001; Rose and Chan, 2016). The destroyed cells and vasculature in burn wounds result in a region of coagulative necrosis with tissue that is not sufficiently oxygenated to support survival or quick healing responses (Rose and Chan, 2016).

It is common practice in the dairy industry to use an aerosol spray or bandage spray with the objective to accelerate wound healing and as prompt treatment of wound infections. In the Netherlands, CTC-spray®, an aerosol spray that contains chlortetracycline, is probably most commonly used for this purpose. As the use of antibiotics increases the selection of antibiotic resistance, the use of antibiotics for several indications is frequently questioned. It may be necessary to use antibiotics on wounds after disbudding, but there is no evidence for this in the literature. Some practitioners and farmers use aerosols without antibiotics, such as Acederm®, Keno™Fix and sprays containing Zinc or Aluminum. Although their somewhat anecdotic observation is that wound healing and infections are similar, we found no systematic studies to support these findings and think that this prevents the farmer and veterinarians from shifting towards a non-antibiotic alternative approach. We decided therefore to perform a clinical study to evaluate the difference in wound healing after disbudding between an aerosol with antibiotics and an aerosol without antibiotics.

MATERIALS AND METHODS

A total of 37 farms from two veterinary practices in the Netherlands was included in this study, the practices were located in the provinces Drenthe and Utrecht. The farms were selected as a convenience sample of those scheduled within a time window of 2 months, agreed with the study design, and fit within the schedule of the students who performed the study. We attempted to include the larger farms with more scheduled dehornings in order to maximize the number of observational units. The disbudding and the observations were done during October and November 2019.

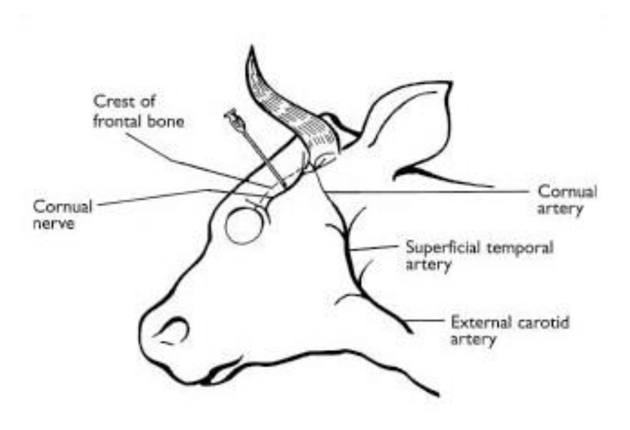


Figure 1. Cornual nerve block (Ames, 2013).

Before the actual disbudding, an existing protocol was used for anesthesia and analgesia. The protocol consisted of an intramuscular injection with xylazine-solution (Sedamun®) in a dosage of 0.20 mg per kilogram body weight of the calf, which was estimated by the veterinarian. Secondly, the calves were injected with meloxicam (Novem 20) in a dosage of 0.5 mg per kg bodyweight for further pain relief. Local analgesia was achieved by injection of 100 mg of a procaine hydrochloride-solution, as a bilateral cornual nerve block (see *Figure 1*). The disbudding was carried out after a waiting period of at least 5 minutes after the corneal nerve block to allow the analgesia to work properly. Disbudding was performed with a butane-fueled cautery dehorner (Portasol III, 17 mm) or an electric dehorner (Lister GmbH, Lüdenscheid, 18 mm). Caution was taken to make sure the contact time of the dehorner with the epithelium was not too long. The horn bud germinal epithelium was removed, as well as the burned necrotic center of the bud.

After disbudding, the two horn buds of each calf were treated with two different sprays. We alternately treated the left and right horn bud with an aerosol spray that contains chlortetracycline (CTC-spray®, Eurovet Animal Health BV) and with a blue aerosol spray without antibiotics, called Keno™Fix. The sprays were applied by spraying from a distance of 15-20 cm for 3 seconds.

At the day of disbudding we collected data on the type of iron (gas or electric dehorner), whether the person that performed the procedure was left or right-handed, whether he or she was a students or a practicing veterinarian, the type of housing (alone or in a group), the hygiene of the pen and whether there was a change that the calf bumped its head while drinking or eating.

EVALUATION OF WOUND HEALING

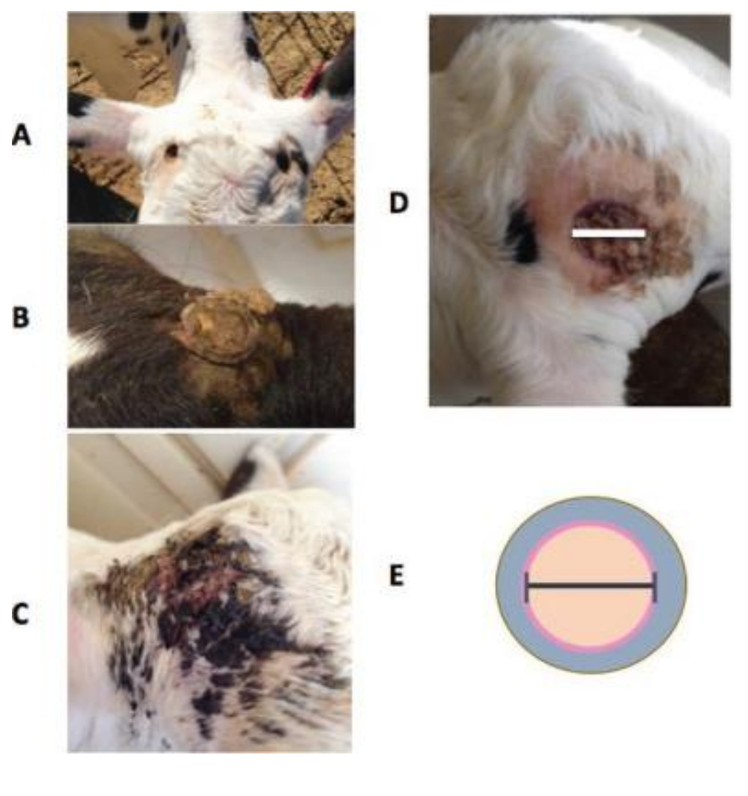


Figure 2. Scoring system, A: LS=1, no scabbing or discharge present; B: LS=2, scabs or raised crusts; C: LS=3, moist or dried purulent discharge; D-E: wound diameter (WD) was measured in millimeters across from the inner edges of the ring were the horn used to be at the widest point (Huebner et al., 2017).

The wound healing of the disbudding site and the degree of infections were considered as primary outcomes and was done twice: on day 6, 7 or 8 and on 27, 28 or 29 after disbudding. The time schedule of the study is shown in *Appendix 1*. The scoring system described by Huebner et al., 2017 was used for the scoring of wound healing. This scoring system is shown in *Figure* 2 and contains 3 different wound scores: LS1= no scab or discharge present. LS2= crusted and scab-filled wound or raised scabs present. LS3= dried or moist purulent exudate. Wound diameter was scored by measuring the inner edges of the budding site at the widest point of this ring. All scores and measurements were done by the two veterinary students. During the study, pictures of different wounds and the corresponding lesion scores were exchanged between students in order to minimize classification bias between the observers.

SCORING OF THE ENVIRONMENTAL FACTORS

Apart from scoring the wounds, the hygiene of the immediate surrounding of the animal (e.g. shed, hutch) was scored as well. Next to that, the type of housing and the risk for accidental head-butts was noted. Scoring was done using a pre-defined scoring system that is shown in *Table 1*.

Table 1. Hygiene scoring system

Score	Description
1	A clean and dry pen with no marks of manure on the walls and a fresh layer of dry bedding present.
2	The pen is clean, but wet. No manure is seen on walls, the layer of bedding is wet, but not soiled by manure.
3	Limited soiling and a wet pen. A few manure marks on the wall and bedding will be scored with a score 3
4	The pen is heavily soiled and wet. Diarrhoea, wet bedding and many manure marks on the wall will be sorted in this category

The risk of head-butting was scored into two categories, based on whether the calf needed to stick its head through an (iron) feeding rack for drinking or roughage intake or not. When all observations were made, both observers sat together to assess the environmental scores by comparing them to the pictures of the housing systems. Small adjustments to the scores were made when a gap between the scorings of both observers was present.

STATISTICAL ANALYSIS

Data was first entered in Microsoft Excel 2016. Type of housing (individual or in a group) and the hygiene score was scored during each visit. The average of the three scores was calculated by the 'Average' function in Excel and used for statistical analysis.

All descriptive and statistical analyses were performed using RStudio version 1.2.1335 (RStudio, Inc., Boston, MA). Results were considered significant when the P-value was ≤ 0.05 and a trend when $0.05 < P \leq 0.10$. To perform the analysis the factor 'time' was converted to the factor 'moment', the scoring at day 6,7 or 8 was fitted as 'week 1' and the scoring at day 27, 28 or 29 was fitted as 'week 4'. To check whether this transformation decreased the quality of the analysis a linear mixed model with the factors 'time' and 'moment' both included was used.

The progress of the WD during the 4 weeks after disbudding for both treatment groups was analyzed using a paired T-test. This analysis was performed on the WD of week 1 and week 4 separately and was also performed on the difference (Δ) between the WD on week 1 and the WD on week 4, calculated for every horn bud individually. To calculate an estimated difference between both treatments and both moments a linear mixed model with explanatory variables 'Treatment' and 'moment' was used. The left-right distribution of the horn pits, whether the dehorner was a student or not, the location (Drenthe or Utrecht), the interaction between the factor 'treatment' and the factor 'moment' were fitted as a fixed effect and a 'calf' and 'farm' were fitted as a random effect. When the AIC of the model was lower when a factor was deleted this model was adopted. If the difference between the AIC of both models was smaller than 2 then the simplest model was adopted. Model assumptions were evaluated by visual inspection of the residuals of the full model by QQ-plots and plotting residuals against predicted values. In the final model, that is shown in *Figure 3* the fixed effects were removed to improve the quality of the model.

```
Linear mixed model fit by maximum likelihood  ['lmerMod']
Formula: WD ~ factor(Treatment) + factor(moment) + (1 | Kalf) + (1 | Bedrijf)
   Data: data

     AIC      BIC   logLik deviance df.resid 
  5197.3   5226.9  -2592.7   5185.3     1014 

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-3.6352 -0.6115 -0.0622  0.5650  5.5247 

Random effects:
 Groups   Name        Variance Std.Dev.
 Kalf     (Intercept) 0.5125   0.7159  
 Bedrijf  (Intercept) 1.4742   1.2142  
 Residual             8.5042   2.9162  
Number of obs: 1020, groups:  Kalf, 255; Bedrijf, 37

Fixed effects:
                     Estimate Std. Error t value
(Intercept)           14.9817     0.2679  55.932
factor(Treatment)CTC  -0.3529     0.1826  -1.933
factor(moment)4       -3.5333     0.1826 -19.348
```

Figure 3. The linear mixed model that has been used to estimate the difference between both treatments

The proportions of the three different LS for both treatments on 1 and 4 weeks post disbudding were analyzed using McNemar's test and a Pearson Chi-squared test. The LS of the disbudding sites were further dichotomized to facilitate the McNemar's tests. Disbudding sites with LS=1 were considered to have normal healing (NH), whereas disbudding sites with LS=2 and LS=3 were considered to have delayed healing (DH). To perform the McNemar's tests the data were displayed in two two-way contingency tables of the observed frequencies of DH and NH at week 1 and week 4, one table for Keno™fix and one for CTC-spray®. The null hypothesis (H0) was that there was no difference between the proportions at both weeks. The H0 was rejected if $P < 0.05$. The Pearson Chi-squared test was used on a 2x3 contingency table of treatment and LS to compare the proportions of the different lesion scores of both treatments with each other. This test was also performed on two tables where the same data where split up in a table for week 1 and a table for week 4.

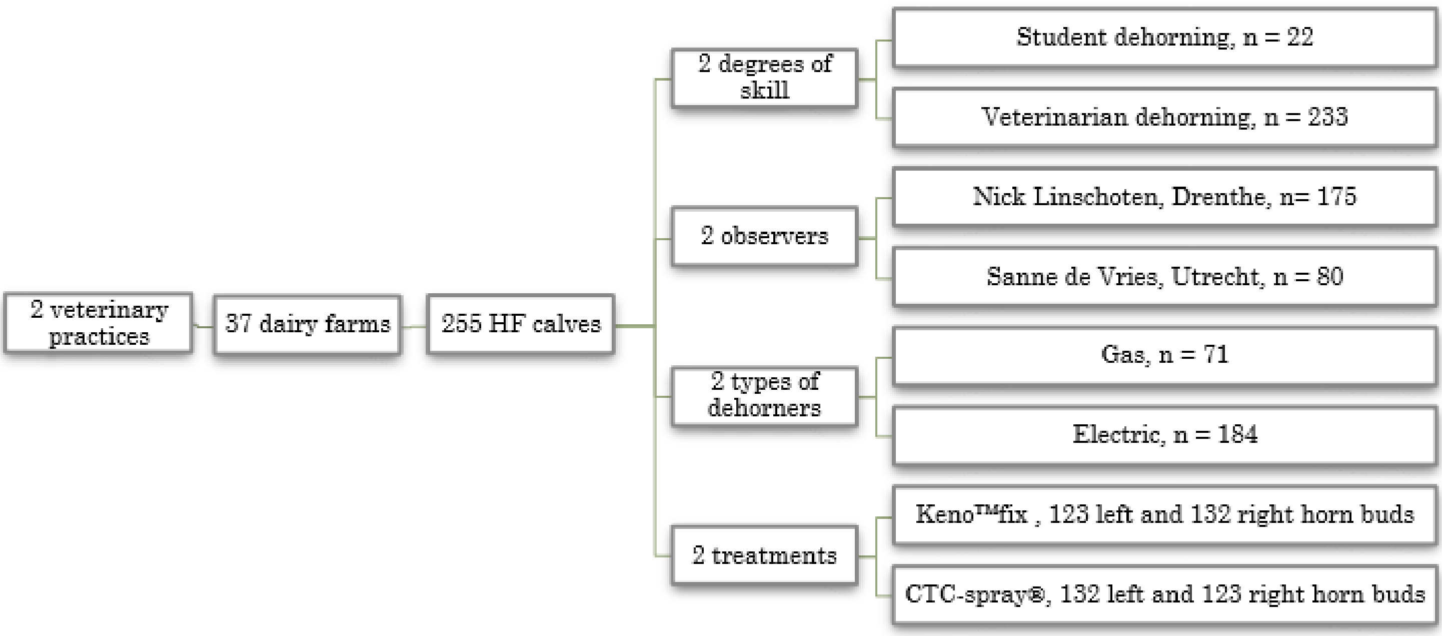
A linear mixed regression model was used to calculate the odds ratio for delayed healing (DH). Rstudio calculated the odds ratio with the formula: $OR = \frac{Odds\ of\ DH\ CTC}{Odds\ \ of\ DH\ Kenofix}$. The following factors where fitted as a fixed effect: The left right distribution of the horn pits, whether the dehorner was a student or not, the location (Drenthe or Utrecht), the interaction between the factor 'treatment' and the factor 'moment'. 'Calf' and farm were fitted as a random effect. The AIC-value, that is an indicator for the quality of the model, was calculated with a Chi-squared test using the drop1 function. When the AIC of the model was lower when a factor was deleted this model was adopted. If the difference between the AIC of both models was smaller than 2 then the simplest model was adopted. In the final model, that is shown in *Figure 4* the fixed effects were removed to improve the quality of the model.

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
 Family: binomial  ( logit )
Formula: I(LSbin == "DH") ~ factor(Treatment) + (1 | Kalf) + (1 | Bedrijf)
   Data: data

     AIC      BIC   logLik deviance df.resid
   946.2    965.9   -469.1    938.2     1016

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9263 -0.4958 -0.3568 -0.2881  3.0433
```

Figure 4. The generalized linear model that has been used to calculate the Odds of delayed healing.

RESULTS

A total of 255 (mainly Holstein Frisian) calves were enrolled in the study. 22 calves were disbudded by a veterinary student under the supervision of veterinarians of the University Farm animal Practice (ULP) or Veterinary center Zuid Oost Drenthe (ZOD). The remaining 233 calves were disbudded by a trained person, such as one of the veterinarians of these veterinary practices or the farmer. On the 37 farms, that were located in the provinces Utrecht and Drenthe, the number of calves included ranged from 1 up to 35. One observer performed all observations in Drenthe, where disbudding was performed with an electric disbudder. The other observer performed all observations in Utrecht, where a disbudder fueled on gas was used most frequently (n=71). We alternately treated the left and right horn bud with both treatments. At the end of the study period Keno™fix was applied to 123 left horn buds and 132 right horn buds. CTC-spray® was applied to 132 left horn buds and 123 right horn buds. The model to find out whether the transformation from the factor 'time' to the factor 'moment' decreased the quality of the analysis indicated that this did not decreased the quality of the analysis, since the AIC indicated that the model was of the same quality when the factor 'time' was left out of the model.



WOUND DIAMETER

The changes in wound diameter for both treatment groups is shown in a boxplot in *Figure 5.*

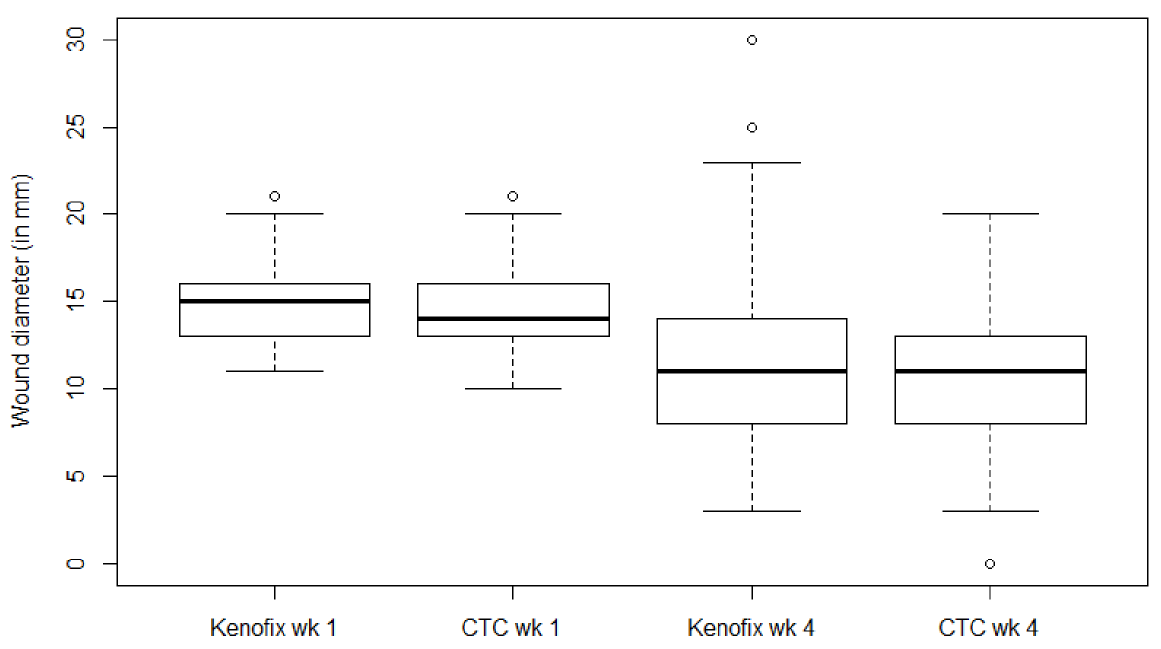


Figure 5. The changes in the mean wound diameter (in mm) at week 1 and week 4 post disbudding. Treatment 1 = Keno™Fix, Treatment 2 =CTC-spray®.

Table 2. Summary of the data on the wound diameter.

	Mean[1]	**SD**[1]	**Min.** [1]	**Max.** [1]
Keno™fix				
Week 1	14.93	2.32	11.00	30.00
Week 4	11.57	4.24	3.00	30.00
ΔWD[2]	3.36	4.90	-15.00	16.00
CTC-spray®				
Week 1	14.71	2.23	6.00	21.00
Week 4	11.02	3.63	0.00	20.00
ΔWD[2]	3.69	4.17	-8.00	14.00

[1]Of the wound diameter (WD), in mm.

[2] ΔWD is the difference in wound diameter, calculated for every horn bud individually (in mm).

In *Table 2* a summary of the data on WD is shown. The mean wound diameter was about 3 mm larger in both treatment groups at week 1 (mean WD = 14.93; SD = 2.32 mm for Keno™Fix, and WD = 14.71; SD = 2.23 mm for CTC-spray®) when compared to week 4 (mean WD = 11.57; SD = 4.24 mm for Keno™Fix, and WD = 11.02; SD = 3.63 mm for CTC-spray®). This significant ($P < 0.001$ for both treatments) decrease in WD shows that wound healing occurs with both treatments. The estimated decrease between week 1 and 4 was 3.53 mm (CI = 3.89 – 3.17 mm) according to the linear mixed model. There was no significant difference between treatments in WD at week 1 post disbudding ($P = 0.11$). The wound diameter was significantly smaller in the group treated with CTC-spray® (mean WD = 11.02; SD = 3.63) when compared with the WD of the group treated witch Keno™Fix (mean WD =

11.57; SD = 4.24) at 4 weeks post disbudding (P = 0.03). The estimated difference between both groups was -0.35 mm (CI = -0.71 – 0.01 mm, P = 0.05) after correction for the factors 'calf', 'farm' and the left-right distribution in a linear mixed model.

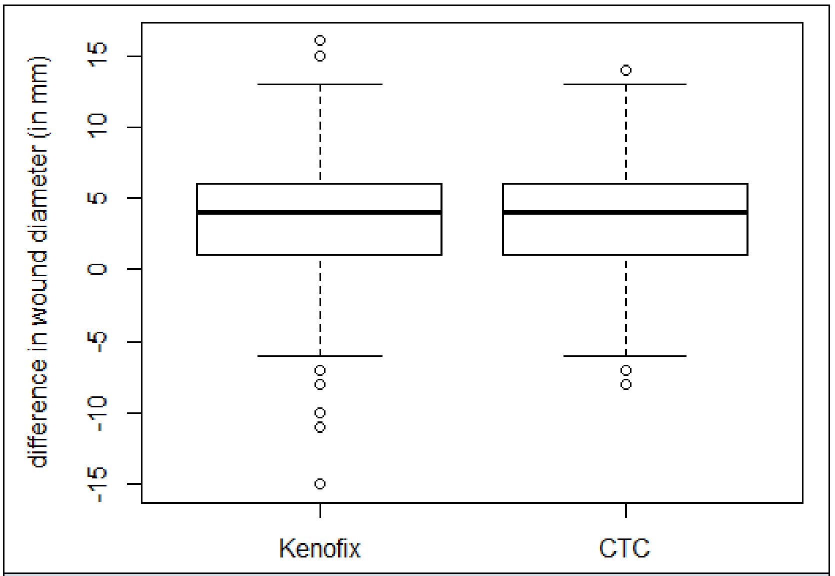


Figure 6. Boxplot of the differences between the WD at week 1 and the WD at week 4 after disbudding, for each calf individually calculated

The difference between the WD in week 1 and the WD in week 4, calculated for every horn bud individually, is shown in *Figure 6* and was not significantly different between treatments (Keno™Fix: ΔWD = 3.36, SD = 4.90; CTC-spray®: ΔWD = 3.69, SD = 4.17; P = 0.24).

The minimal ΔWD (*Table 2*) is negative in both treatments; this means that the WD was larger at week 4 when compared to week 1. In 80 of the 510 calculated ΔWD of the horn pits the outcome was negative. Approximately 70% (56/80) of these negative ΔWD also had a LS of 2 or 3. The correlation between the WD and the LS, shown in *Figure 7*, shows that wounds with a larger diameter tend to have a high LS as well. Some differences were observed between both locations: Utrecht had a mean WD of 12.6, with a mean LS of 1.3, while in Drenthe the mean WD was 13.2 with a mean LS of 1.2

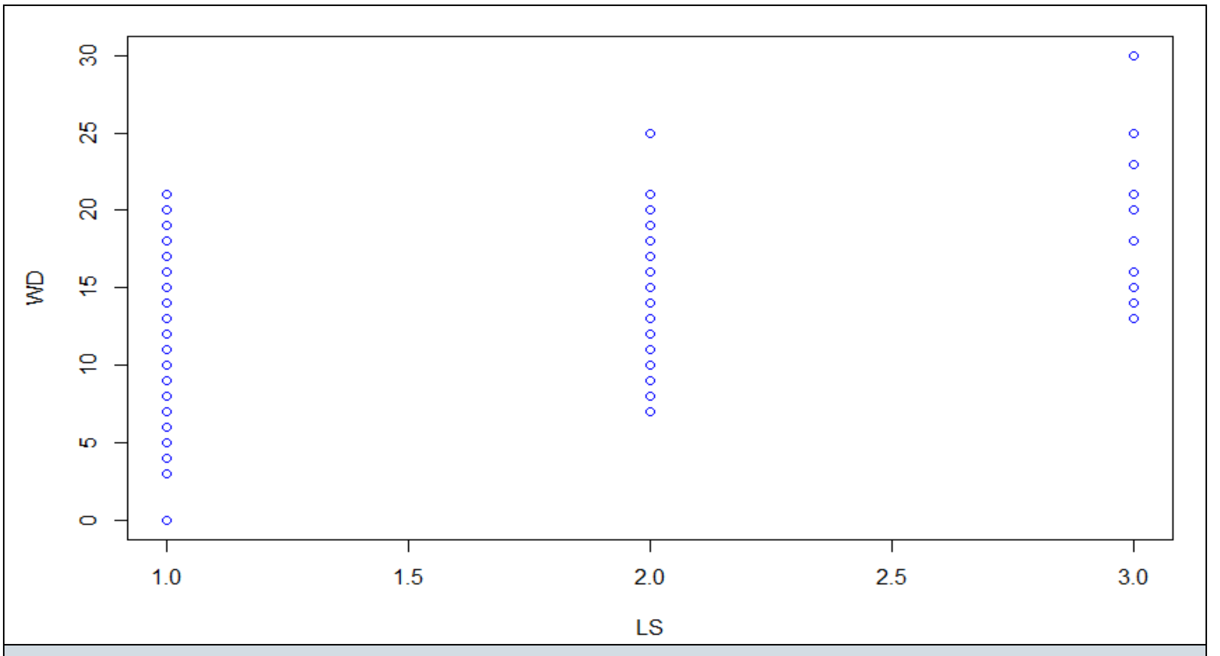


Figure 7. The correlation between the wound diameter (WD) and lesion score (LS).

LESION SCORE

Pictures of a typical appearance of the different LS at both scoring moments are shown in *Figure* 8. At week 1 post disbudding wounds that were treated with Keno™Fix had in general an aspect that was more wet than wounds that were treated with CTC-spray®. Therefore, these wounds were more likely to get a higher LS. Four weeks after disbudding most wounds were already closed, so we decided to score wounds with large crusts (>20 mm) also with a score 3. The proportion of calves with different LS for both groups at 1 and 4 weeks after disbudding is shown in *Figure 9*.

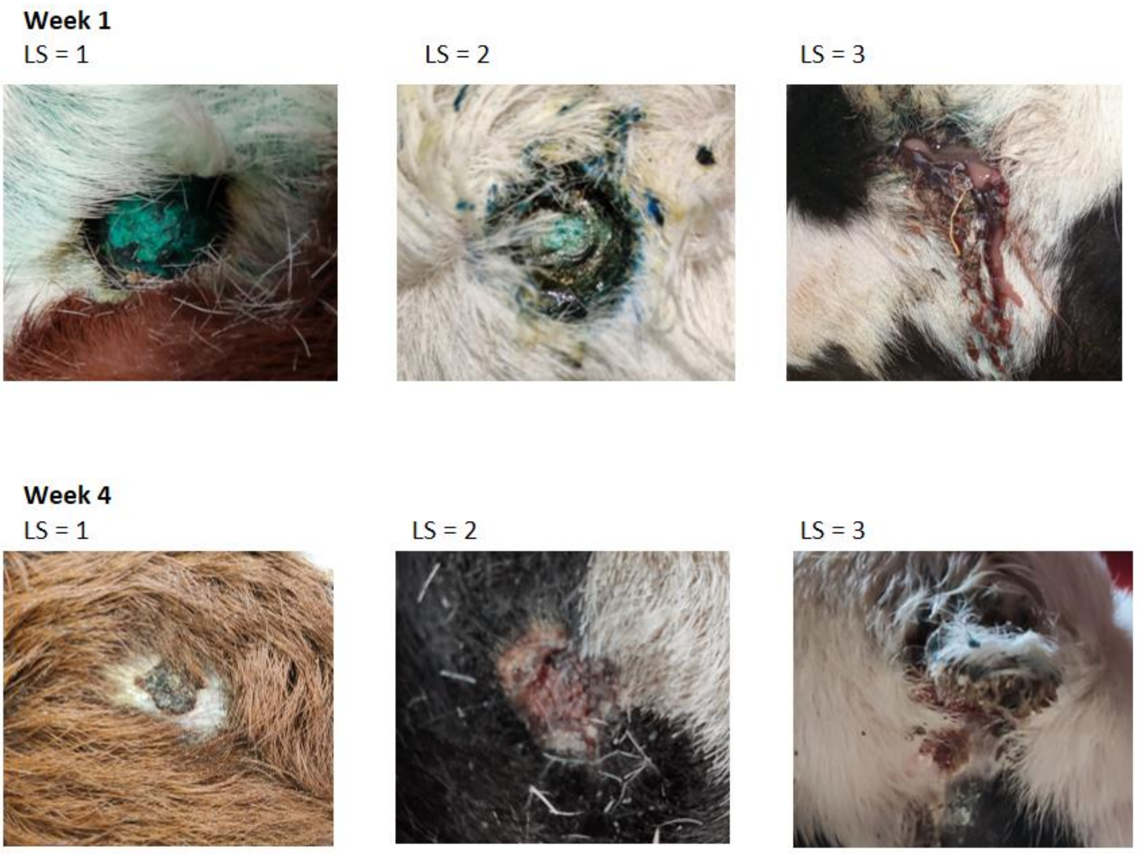
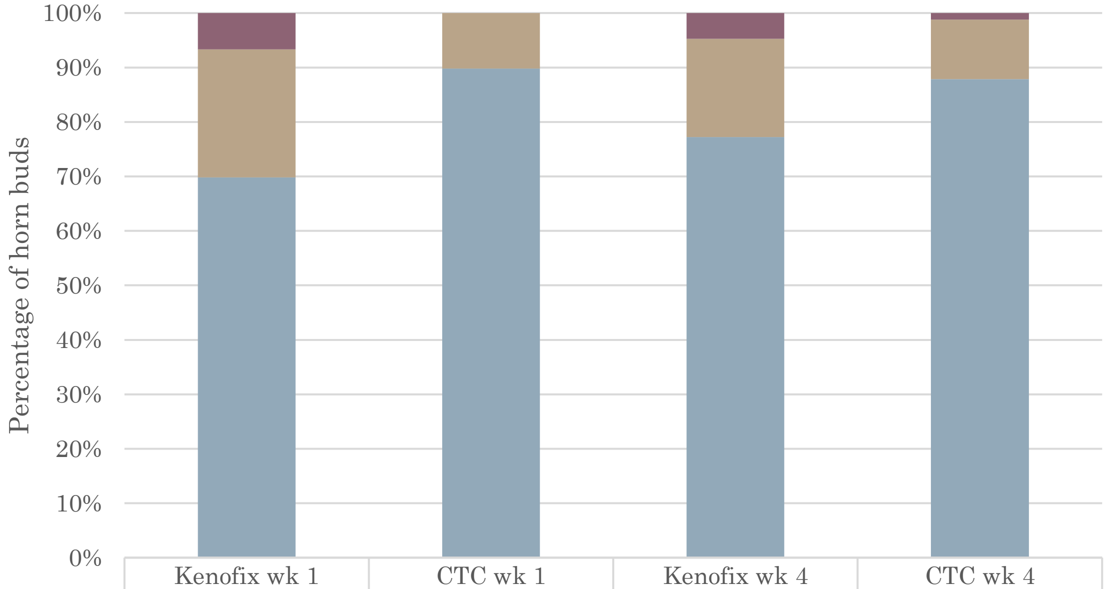


Figure 8. Pictures the different LS at both scoring moments.



	Kenofix wk 1	CTC wk 1	Kenofix wk 4	CTC wk 4
LS = 3	17	0	12	3
LS = 2	60	26	46	28
LS = 1	178	229	197	224

Figure 9. Proportion of horn buds at week 1 and week 4 with different lesion scores (LS) by treatment groups: Keno™Fix and CTC-spray®. In the table below the absolute number of horn buds with different LS are shown. LS = 1: no scab or discharge present, LS = 2: crusted and scab-filled wound or raised scab present, and LS = 3: dried or moist purulent discharge.

The proportions of the disbudding sites with LS = 3 and LS = 2 are numerically higher in de Keno™fix group when compared to the CTC-spray® group at both scoring moments (*Figure 6*). At week 1 we observe 7% versus 0% for LS = 3 and 24% versus 10% for LS = 2, respectively, and at week 4 we observe 5% versus 1% for LS = 3 and 18% versus 11% for LS = 2, respectively. Testing whether there is a difference between the proportions of the three lesion scores of both treatments with the Pearson Chi-squared test resulted in a significant difference between both treatments ($P < 0.001$). When analyzing the difference in LS proportion among the treatment groups at both moments separately, a significant difference was found at both moments ($P < 0.001$ at week 1, $P = 0.007$ at week 4).

The McNemar's test that compared the proportion of DH and NH at week 1 post disbudding with the proportion of DH and NH at week 4 post disbudding, showed that the proportions seemed to differ for Keno™fix ($P = 0.06$). For CTC-spray® the proportions of DH and NH are the same at both moments ($P = 0.48$).

Logistic regression models were used to calculate the odds ratio (OR) between the odds of DH for both treatments. The OR, that Rstudio calculated with the formula:

$OR = \frac{Odds\ of\ DH\ CTC}{Odds\ of\ DH\ Kenofix}$, was 0.4 (CI: 0.3 – 0.5) for CTC-spray® ($P < 0.001$). This means that the odds of DH was 2.5 times higher for Keno™fix than the odds of DH for CTC-spray®. *Table 3* shows that there was no significant difference in the odds for DH when the unadjusted scores were calculated for 'moment' and the left/right distribution of the horn buds. The odds for DH was 2.5 times higher for the wounds that were dehorned by students (P = 0.005) and was 1.67 times higher for wounds that were dehorned in Utrecht, thus, scored by observer 1 (P = 0.05).

Table 3. Unadjusted scores resulting from logistic regression models of delayed healing

Factor	Total	LS=1 Prop[1]., n	LS=2 Prop[1]., n	LS=3 Prop[1]., n	Odds ratio	95% CI	P-value
Treatment							
Keno™fix	510	75%, 380	19%, 100	6%, 30	Ref.		
CTC-spray®	510	88%, 450	11%, 57	1%, 3	0.4	0.3 – 0.5	<0.001
Moment							
Week 1	510	80%, 408	17%, 84	4%, 18	Ref.		
Week 4	510	82%, 422	14%, 73	3%, 15	0.8	0.6 – 1.1	0.24
Left/Right horn bud							
Left	510	82%, 420	15%, 75	3%, 15	Ref.		
Right	510	80%, 410	16%, 82	4%, 18	1.1	0.8 – 1.6	0.40
Dehorning person							
Student	88	68%, 60	24%, 21	8%, 7	Ref.		
Veterinarian	932	83%, 770	15%, 136	3%, 26	0.4	0.2 – 0.8	0.006
Location							
Utrecht	320	77%, 246	18%, 58	5%, 16	Ref.		
Drenthe	700	83%, 584	14%, 99	2%, 17	0.6	0.4 – 1.0	0.05

[1]The proportion of horn buds with a certain LS, for the calculating of the odds ratio the proportion of DH and NH were used.

DISCUSSION

The goal of this study was to evaluate the difference in wound healing between an aerosol spray with antibiotics and an aerosol spray without antibiotics. Most tests on WD did not indicate a difference between both treatments. However, the T-test on week 4 and the linear mixed model did show a significant difference between both treatments in the favor of CTC-spray®. The estimated difference between the treatments in WD was 0.35 mm with a SE of 0.18 mm. This difference is too small to be of clinical importance.

The results of the Chi-squared tests and the logistic regression models show that wounds that were treated with Keno™Fix were more likely to have DH (LS = 2 + LS = 3). Since a higher LS is correlated with a larger WD it is expected that the tests on WD would also indicate a difference between both treatments. A possible explanation for the fact that no difference in WD was found in the comparison of both treatments, is that wounds treated with Keno™fix and got a high LS recovered quicker from this, than the wounds that where treated with CTC-spray® and got a high LS. Another explanation is that the wounds that had LS = 1 and were treated with Keno™fix decreased faster in

WD than the wounds with LS = 1 that where treated with CTC-spray®. The scoring system used focused on the degree of inflammation present. One week post disbudding the wound healing did not achieve the maturation and remodeling phase yet. Most wounds were still in the inflammation phase and some in the proliferation phase. Therefore, the scoring system seemed to be an accurate representation of the wound healing. But it is questionable whether a high LS is actually something that causes delayed healing, since inflammation is a normal stage in wound healing. Excessive inflammation is an indicator of infection, and LS = 3, which includes wounds with moist or dried purulent discharge, definitely indicated infection. LS = 2 included scabs or raised crusts, which do not certainly indicate infection. Therefore, the OR of 2.5 is possibly an overestimation.

At four weeks post disbudding, the majority of the wounds were in the maturation and remodeling phase. During this phase contraction of the wound occurs (Rose and Chan, 2016). In the scoring system that was used, a wound with more contraction, meaning it was more healed, was given the same score as a wound with the same diameter that was less contracted, since we did not score the degree of contraction, but measured the WD at the widest point of the wound. Beside wound contraction, other factors should have been added to the scoring system at week 4: the degree of epithelialization and whether the necrotic crust had fallen off or was still attached.

The McNemar's test showed no difference between the proportions of the different LS in both weeks. This can be explained by the inability of the wounds with LS = 2 and LS = 3 at week 1 to move forward to the next stage of wound healing. However, since the McNemar's test calculates proportions, it is possible that the wounds with an LS = 2 or LS = 3 at week 4 are other wounds than the wounds with these scores at week 1. Besides that, we also decided to score wounds with large crusts (>20 mm) at week 4 also with a score 3. The latter also causes a deviation in the proportions of the lesion scores.

In 80 of the 510 cases, we observed that the WD was larger in week 4 compared to week 1. Since 70% (56/80) of these cases had a lesion score of 2 or 3, we suggest that the increase in WD may be related to an inflammatory disruption in the wound healing. It is possible that the remaining 30% of the disbudding wounds with a negative ΔWD were also caused by inflammation, which was already resolved at the scoring moment, because there were three weeks between the two scoring moments. The time schedule of the observing students did not allow them to also score at weeks 2 and 3, and in the discussion of Huebner *et al.* it was stated that they would like to have measured and scored the wounds for one or two more weeks, than the three weeks they did (Huebner et al., 2017). Therefore, in this experiment, the wounds were scored on week one and week four.

The scoring was done by two veterinary students at two locations. A small difference in mean WD and mean LS was observed between them. Observer 1 (location = Utrecht) had a relatively low WD with a high LS, whilst it was the other way around with observer 2 (location = Drenthe). It is to be expected that there is a correlation between the WD and the LS. The fact that that is not present in this study suggests that there is a deviation in the scoring of both observers. This could possibly be explained by the factor 'type of dehorner', as most wounds that were scored by observer 1 were created by the disbudding with a gas dehorner and observer 2 did not observe any wounds that were disbudded by a gas dehorner. But the fact that Adcock and Tucker did not find a difference in wound healing when they compared a gas dehorner with an electric dehorner contradicts this (Adcock and Tucker, 2018). Therefore, a slight difference between the observations of both students or the locations might interfere with the results.

Adcock *et al.* describes whether the type of dehorner has an influence on wound healing. It was found that there is a difference in wound healing between the left and the right horn (Adcock et al., 2019). Therefore in this experiment the treatments were randomly applied to the left and the right horn bud. This was accomplished by spraying Keno™fix on the left horn bud and CTC-spray® on the right horn bud of the fist calf that was disbudded, with the second calf the sprays were applied the other way around, and so on. This is not completely random, but starting side of the treatments differed between farms and sometimes the calves where changed pens between visits. Therefore, and because of the blue color of both sprays, the scorer did not know which spray was applied on which side at the next visit, so there was no bias. The distribution of the two treatments to the left and the right horn buds was not even. This might have caused a slight difference in the outcomes of the T-tests, Chi-square and McNemars tests, but was corrected in the linear mixed model and the logistic regression model.

The exact mechanism of action of Keno™Fix is currently unknown. It does not contain any antibiotics but the producers claim that it forms a "second skin", which creates a strong and flexible film on the skin that releases a constant and prolonged biocidal substance. The label does not mention that repeated appliance is necessary. CTC-spray® can be prescribed for the treatment of superficial traumatic or surgical wounds. Since the active ingredient of CTC-spray®, chlortetracycline, is an antibiotic, there is stated in the summary of product characteristics (SPC) that unnecessary use should be avoided to prevent antibiotic resistance. For the treatment of superficial traumatic or surgical wounds, contaminated with germs sensitive to chlortetracycline, a single treatment is recommended.

A lot of flies were present at some farms. This might have interfered with the wound healing, but is corrected for in the models with the random factor 'calf'. Another potential factor that might have interfered with the wound healing is the person that executes the dehorning procedure. Every farmer, veterinarian or student has another technique, which might differ in the total time they take burning the wound, the way they remove the pit and how large and deep the wound is after the procedure. All of these factors might also differ between the left and the right side of the same calf. Research in humans shows that the dept of a burn wound is a predictor of healing time (Merz et al., 2010). A correction for the latter could have been performed, when a measurement of the wound diameter right after the dehorning procedure wound have been made. However, for every calf the person dehorning was the same for the left and right side. In this experiment the cauterized horn bud tissue was removed, because research shows that it is advantageous to remove the horn bud tissue after cautery disbudding to increase efficacy of wound healing and prevent infections (Sutherland et al., 2019).

The farms used were representative for other farms in the Netherlands, since a lot of different types of housing were used and the differences in farm size and hygiene scores on the farms were large. Also, the farms were located in two very different regions in the Netherlands. This results in a high external validity. To improve the external validity of the research further, the experiment should be repeated during other seasons. Especially the warm temperatures during the summer might interfere with wound healing, because a lot of flies are present during this season.

CONCLUSIONS

This study found no difference of clinical importance in wound healing between the application of the topical wound bandage spray Keno™Fix and CTC-spray® when measuring the wound diameter after cautery disbudding. Wounds that were treated with Keno™Fix had a greater chance to get a high lesion score, than wounds that were treated with CTC-spray®. But, since this is not combined with a difference in wound diameter, it is arguable whether the higher LS is really a problem that causes delayed healing, or whether it is a normal stage of wound healing. The decision to use one of both sprays depends on the goals of the veterinary practice and the farmer, considering that we want to decrease the use of antibiotics, but the surface of the wound will be not as good looking, when we do.

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APPENDIX

APPENDIX 1: TIME SCHEDULE

The practical part of the study started on 14 October 2019. The time schedule of the gathering of the results is presented in the table below.

Time schedule of the gathering of the results		
Week 2 (14/10/2019 – 18/10/2019)	Tue, Thu: Dehorn group A and fill in checklist	
Week 3 (21-10-2019 – 25/10/2019)	Tue, Thu: Dehorn group B and fill in checklist	Wed: Scoring 1 of group A
Week 4 (28-10-2019 – 01/11/2019)	Tue, Thu: Dehorn group C and fill in checklist	Wed: Scoring 1 of group B
Week 5 (04/11/2019 – 08/11/2019)	Tue, Thu: Dehorn group D and fill in checklist	Wed: Scoring 1 of group C
Week 6 (11/11/2019 – 15/11/2019)	Wed: Scoring 2 of group A	Wed: Scoring 1 of group D
Week 7 (18/11/2019 – 22/11/2019)	Wed: Scoring 2 of group B	
Week 8 (25/11/2019 – 29/11/2019)	Wed: Scoring 2 of group C	
Week 9 (02/12/2019 – 06/12/2019)	Wed: Scoring 2 of group D	