Infrared Thermography for use as early Detection Method for Digital Dermatitis in Dairy Cattle

Master Thesis of Research Traineeship

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ABSTRACT

Digital dermatitis (DD) is widely prevalent in cattle in the world and causes economic losses as it can cause lameness and a decreased milk yield. For adequate treatment, proper diagnostics are needed, which are preferably cost-effective and least labour intensive to assure producer implementation. Therefore a new, non-invasive way of detecting DD might be rewarding, such as infrared thermography (IRT), which detects heat as related to inflammation. The aim of this study was to investigate the use of infrared thermography (IRT) in the detection of DD in dairy cattle. The hypothesis was that a DD lesion goes with inflammation of the skin of the bulb of the heel and therefore radiates more heath than a not affected foot. A total of 850 cows (four farms visited five times, one farm visited once) were scored for DD, lameness and IRT images were taken before (pre-) and after (post-) hosing of the hooves. This resulted in 3,314 images, divided over two different IRT cameras: a cost-friendly and an expensive one. The means of the maximum temperatures from the images per DD stage were compared to each other and a statistical significance was found for the difference in the mean of the maximum temperatures between M0 and M2 stages for both pre and post images. Also, the differences in mean temperatures between absence of DD (M0) versus active DD lesions (M1 and M2), absence of DD versus chronic DD lesions (M3 and M4) and chronic versus active DD lesions were significantly different for pre images. For the post images only the mean temperatures for the absence of DD versus active DD lesions were significantly different. A sensitivity of 0.500 and a specificity of 0.697 was found in establishing a cut-off temperature to detect lameness using IRT on hind feet, which has therefore a limited value in lameness detection. Also there appeared to be a very strong correlation between the two cameras, meaning that the more expensive camera didn't exceed the results of the cost-friendly camera and a strong correlation between pre- and post-images was found, which tackles the need of washing of the hooves. In conclusion, IRT was able to find significant differences between the mean maximum temperatures of the M stages of DD which is promising for a developing new method of early DD detection. Also the results support a future on-farm implementation as the cost-friendly camera performed equally well and hosing of the hooves didn't improve any results and is therefore not adding extra labour.

Keywords: Dairy cow, digital dermatitis, infra red thermography, diagnostics

INTRODUCTION

Digital dermatitis (DD) is an infectious foot lesion in cattle and characterized by inflamed skin, mostly of the bulb of the heel (Holzhauer et al., 2006; Biemans et al., 2017). It was discovered in Italy by Cheli and Mortellaro in 1974 but nowadays it has a worldwide occurrence (Holzhauer et al., 2006; Scheirlinck, 2011; Kissels, 2016). However the prevalence of foot lesions in general and DD in specific, varies among countries, regions and housing systems (Solano et al., 2016), it has come to an endemic stage in several countries among which countries in Europe (the Netherlands (Holzhauer et al., 2006) and the United Kingdom (Clarkson et al., 1996)) and in North America (the United States of America (Wells et al., 1999) and Canada (Solano et al., 2016)). In Alberta, Canada, 15% of dairy cows and 94% of dairy herds that are trimmed on whole-herd basis are affected (Solano et al., 2016). Digital dermatitis is known for its negative impact on animal welfare, productivity and reproductive performance (Holzhauer et al., 2006; Cramer et al., 2018) as it can cause lameness, pain and discomfort for a cow (Holzhauer et al., 2006; Bruijnis et al., 2012;). This eventually leads to economic losses and increased labour for the producer (Bruijnis et al., 2012; Mülling et al., 2014). The costs of all foot disorders combined on a farm can add up to $ USD 7001 annually with an average of $ 4899, based on a 65-cow farm (Bruijnis et al., 2010). In addition, they showed that DD accounted for the highest part of these losses because of its high prevalence (Bruijnis et al., 2010). However, in different housing systems, other foot lesions may account for the highest part of all losses, as for example non-infectious causes are more likely to occur (Cha et al., 2010; Charfeddine & Perez-Cabal, 2017).

To create and maintain adequate hoof health management, proper diagnostics of foot lesions are needed to allow for early identification and treatment (Jacobs et al., 2018). Concerning DD, diagnoses are usually made macroscopically either in a trimming chute or in the milking parlour (Relun et al., 2011; Biemans et al., 2017; Oliveira et al., 2017), considering the use of the trimming chute as the reference (Oliveira et al., 2017; Solano et al., 2017; Cramer et al., 2018). Several studies suggest that producers not always have the ability to correctly diagnose hoof lesions (Dutton-Regester et al., 2018), which is why they rely on hoof trimmers. Even though using the trimming chute might be the most reliable way to score DD, it is not very efficient regarding a regular inspection of the hooves as it is labour-intensive and takes a lot of time (Oliveira et al., 2017; Cramer et al., 2018). Also, trimming usually is not performed on regular basis that all early-onset DD can be detected which is essential to allow for an early treatment (Solano et al., 2017; Dutton-Regester et al., 2018). In addition, getting trimmed might be a stressful experience for cattle (Thomsen et al., 2008; Solano et al., 2017).

When it comes to scoring lesions in the milking parlour, washing of feet is highly recommended before starting to score (Oliveira et al., 2017). A study with 22 dairy herds in Denmark showed that scoring DD in cows with washed feet resulted in 32 % more cases being detected, compared to when the feet weren't washed (Oliveira et al., 2017). Therefore, many cases could be missed and thus not treated if not washed at parlour inspection. Nevertheless, washing isn't always done, among other things due to a cow's discomfort or fear of udder contamination in the milking parlour (Oliveira et al., 2017). Also, non-lactating heifers as well as dry cows, which can also be affected by DD, are missed when scoring in the milking parlour is the main method for diagnosing DD (Oliveira et al., 2017). However, it is a proper diagnostic method to only determine presence or absence of DD (Stokes et al., 2012b; Oliveira et al., 2017). A study which reviewed different methods of detecting hoof lesions, including DD, came to the conclusion that the sensitivity and specificity varies widely between different methods (Dutton-Regester et al., 2018). They found that sensitivity and specificity for visual detection of DD in the milking parlour ranged from 0.60 to 1 and from 0.63 to 1, respectively.

Digital Dermatitis lesions are usually identified using the M-stages of Döpfer (Dopfer et al., 1997; Berry et al., 2012; Mülling et al., 2014), see Table 1 and Figure 1.

CLASSIFICATION	DISEASE STAGE	CHARACTERISTICS
Stage M0	Healthy claw	- Macroscopically unaffected digital skin
Stage M1	Early/Subclinical	- Active ulcerative/granulomatous, circumscribed lesion - < 2 cm in diameter
Stage M2	Acute/painful	- Ulcerative, active lesion - > 2 cm in diameter

Stage M3	Healing	- Scab-covered lesion - Firm surface - Often seen after treatment with antibiotics - Not painful on manipulation
Stage M4	Chronic	- Hyperkeratotic/proliferative lesions - Raised surface with different-size growths (from filamentous to mass proliferation: hairy warts)
Stage M4.1	Chronic active	- Presence of M4 and M1 - Small M1 lesion within a hyperkeratosis - Painful

Table 1: Description of different stages of Digital Dermatitis as described by Döpfer et al. (1997) *and extended by Berry et al.* (2012).

Figure 1 (Zinicola et al., 2015)*: Examples of the different M-stages as made by Döpfer et al.* (1997) *and Berry et al.* (2012).

The M1, M2 and M4.1 DD lesions are considered active (Jacobs et al., 2018) and associated with playing a role as reservoir for the infectious specimens (Orsel et al., 2018). Therefore they play a key role in the treatment and prevention of DD on farm (Mülling et al., 2014).

As stated above, all different methods of detecting DD go with several disadvantages. Therefore, a method to quickly and easily diagnose DD, will be rewarding (Alsaaod & Buscher, 2012) when there is no need to wait for the next trim. One possible way for assessing DD can be infrared thermography (IRT), which has various applications in human and farm animal as well as in equine medicine (Alsaaod & Buscher, 2012; Soroko & Howell, 2018). For farm animals it has been used among other things for monitoring udder health status (Zaninelli et al., 2018), monitoring health and welfare in dairy cows at a distance (Stewart et al., 2017) and for detecting foot-and-mouth disease on feet of cattle (Rainwater-Lovett et al., 2009). This method is based on infrared radiation, which is emitted by all objects, depending on their temperature. This radiation can be captured by thermal (infrared) cameras (Eddy et al., 2001). These cameras detect an objects surface temperature, which in the case of detecting DD would be the skin of the extremity (Eddy et al., 2001; Alsaaod & Buscher, 2012). The temperature of the skin is highly dependent on the temperature of the underlying tissue and circulation. Variation in skin temperature, captured by an IRT camera, could then be related to underlying inflamed tissue or changed metabolic activity (Alsaaod & Buscher, 2012), as might be the case in DD. Therefore the objectives of this study were to determine if IRT can be used to detect DD lesions that are visibly identifiable (1), to determine if different stages of DD lesions can be distinguished using IRT images (2), to determine if lameness is related to inflammation in the hoof as measured by IRT (3), to determine if the use of a cost-friendly camera would obtain the same results as a more expensive camera (4) and finally to determine if there is a difference between IRT images made before and after hosing of the hooves (5).

MATERIALS AND METHODS

Farm and Cow Selection

The farms used in this study had to meet the following criteria: only Holstein-Friesian cows and housed in free stall barns. Five farms were selected and each of them was located in Alberta, Canada. Four of these farms were visited five times with an interval of three weeks, within the period of May – August 2013, and one was visited only once, in November 2013. For all of the four farms, the five visits were consistent in the AM or PM milking time, but there was variation in what milking time was attended per farm. At the first farm visit about 40 cows were randomly selected in the milking parlour. The same cows were used for all of the following visits, with a small drop-out as a result of, among other things, dry-off. Two IRT images were captured of the back of each hind foot, focusing on the pastern; one before and one after hosing of the feet. This resulted in four images per cow at each visit. Only hind feet were used, as more than 80 % of DD lesions occurs in the hind feet (Read & Walker, 1998). While in the milking parlour, the cows were visually scored for DD after washing the feet, using a mirror and LED headlamp for a better view. This was recorded together with the cow ID and IRT image number. On the same farm visit the locomotion of the selected cows was scored using the 5-point scoring system, where a score 1 is considered not lame and a score 5 is considered severely lame (Sprecher et al., 1997). Also, to compare a cost-friendly IRT camera (camera I) to a more expensive one (camera II), the one farm that was only visited once was used to investigate the differences between these cameras. Therefore, another two images per foot per cow were taken with camera II before and after hosing of the feet. An overview of the used farms and the number of images that they generated is found in the results.

IRT Software

For this study two different IRT cameras were used: camera I was a FLiR Systems Inc., i3 thermal imaging camera and camera II was a Testo 875 Thermal imager (875). Both were so-called handheld cameras which didn’t allow for images to be taken from a fixed position, but every image was taken from about 0.5 meter distance. At the beginning of every farm visit, the ambient temperature was taken, to calibrate the camera for the environmental temperature. The cameras and their software are described below. The images were uploaded in the matching software and analysed one by one. The results were combined in an Excel 2013 spreadsheet. Every image that was unclear which showed the foot under a different angle was discarded.

Camera I

This camera costs about 1550 CAD nowadays and runs on the ThermaCAM Researcher Professional 2.8 SR-2 software to analyse the images. This camera was able to give information about an image as a whole, and about a selected part of an image. This was important for this study as the interesting part of the image is the place on the leg where DD usually occurs. This could be accomplished by manually drawing a box using a selection tool. The software then provided information about both the whole image and the box that was drawn, see Figure 2 and 3. This box was drawn for every image separately, covering the pastern from the lowest point of the dew claws down to the lowest part of the heels. This was to prevent interference of temperature of the ground or the leg above the dew claws. The temperatures that are reflected by different colours are presented in the legends, next to the images. Camera I was the main camera, which was used for every objective.

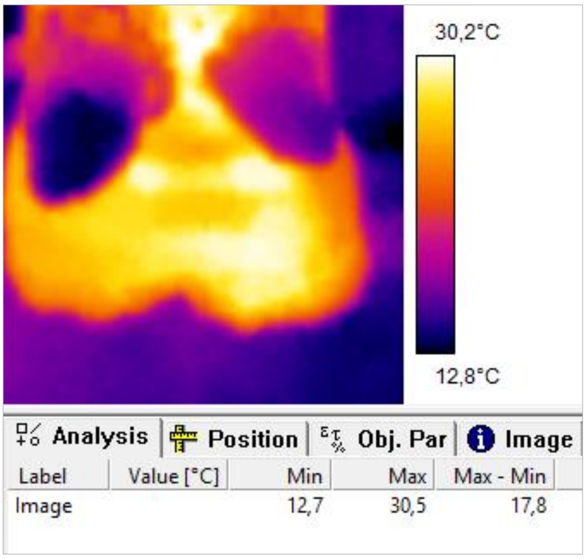

Figure 2: IRT image from ThermaCAM Researcher software. 'Min', 'Max' and 'Max-Min' stand for minimum temperature, maximum temperature and maximum temperature minus minimum temperature respectively. All values are measured over the whole image.

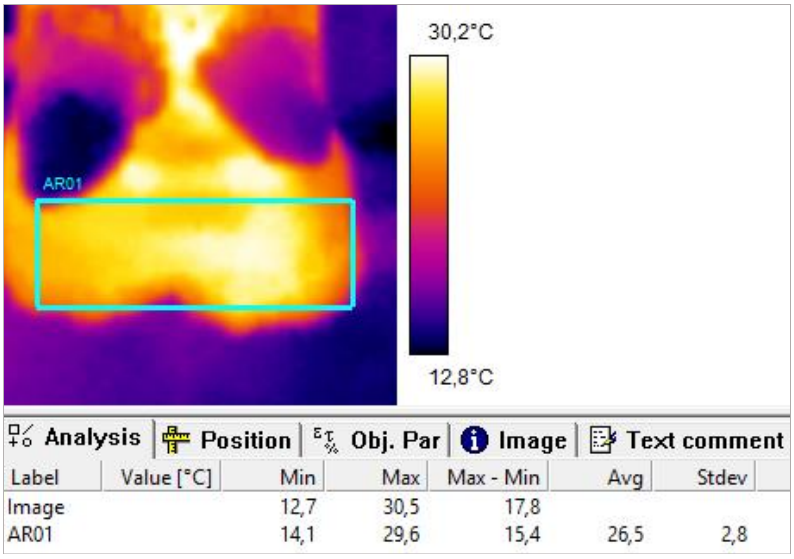

Figure 3: IRT image from ThermaCAM Researcher Software. The green box provides extra information, shown in the AR01 row in the table below the image. 'Avg' and 'Stdev' stand for average temperature and standard deviation respectively. The values in the row 'Image' are measured over the whole image.

Camera II

This camera costs over 3,000 CAD nowadays, depending on the model, and runs on Testo IRSoft 4.3 software. This software disposed of the same selection tool to draw a box at the relevant part of the image. The boxes were drawn using the same criteria as described for the other camera. The program provided information about the box, but not about the image as a whole, see Figure 4 and 5. Camera II was only used for the fourth objective, to compare the two cameras. The purple or blue colours represent colder temperatures compared to the yellow colour which represent the hottest parts of the image, as there is no legend represented in figures to indicate that. Camera II was only involved in the objective for the comparison between the two cameras.

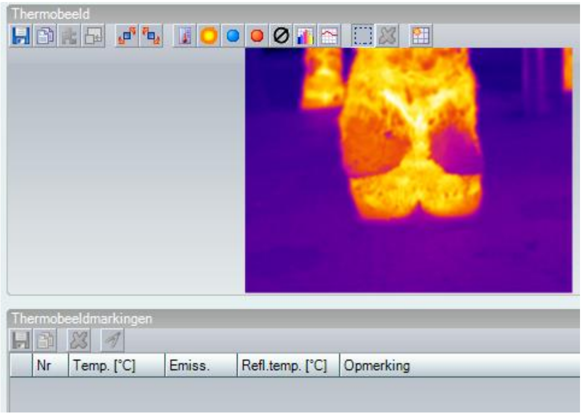

Figure 4: IRT image from Testo IR Soft 4.3 software. The program provides no information over the image as a whole.

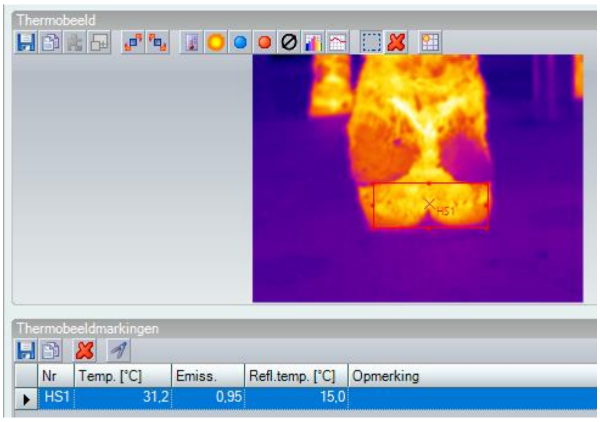

Figure 5: IRT image from Testo IR Soft 4.3 software. The red cross in the shows the hottest spot in the red box (HS1).

Statistical Analysis

Excel 2013 was used to combine the outcomes of the IRT images analysis with other variables, such as farm, number of the visit, cow number, DD score and lameness score. Then, XLStat and Stata15 were used to perform most of the statistical analysis. Below the different statistical tests will be described per objective. For every objective, the variable 'maximum temperature of the hoof' was used and missing data were excluded.

Objective 1: Determine if IRT can be used to detect DD lesions that are visibly identifiable

For this objective, the difference in maximum temperature of the hooves of cows having DD and cows not having DD needed to be established. This was done by running independent t-tests between the means of the maximum temperatures of M0 and M2 stages for both before hosing (pre-) and after hosing (post-) images to see if they were significantly different. Also, t-tests between the means of M2 versus all of the other stages together were performed and between the means of M0 versus all of the other stages together, to determine if the M2 lesions and M0 stages differ significantly from the other DD stages. M2 lesions were taken apart as they are very relevant regarding early detection as they are acute and painful (Mülling et al., 2014; Orsel et al., 2018) and need to be treated to prevent further spread of the disease (Mülling et al., 2014).

Then, a threshold temperature needed to be determined to establish the distinction between hooves with DD and hooves without DD using IRT. To determine this threshold temperature, XLStat was used to produce several ROC curves and to calculate the matching 'Area Under the Curve' (AUC). These curves compare two possibilities (positive and negative, for example having DD and not having DD) with a continuous variable: the maximum temperature of each hoof. The calculation provided both a graph (the ROC curve) and a table that shows all combinations of sensitivity and specificity for each possible threshold temperature and indicates the optimum temperature of the highest or optimum combination of sensitivity and specificity. This temperature should be interpreted as the best possible 'cut-off value' above which all cases are considered as positive (having DD) and below which all cases are considered as negative (not having DD). The cut-off value preferably has a high true positive rate and a low false negative rate, which is described by the AUC. This value is used to describe the ability of the test (here the IRT camera) to distinguish between the both categories. A perfect test will have an AUC of 1 and a test that has no better results than chance will have an AUC of 0.5 (Petrie & Watson, 2013).

Also, a total of six ROC curves were constructed for all of the cows, divided over two categories: pre- and post-hosing. For this, data from left and right feet were combined in a cow-level score. M0 was considered negative and all other M stages were considered positive for the first ROC curve (Method 1) of both categories (pre- and post-hosing). M0, M3 and M4 were considered negative and M1 and M2 were considered positive for the second ROC curve of both categories (Method 2), as those are the stages characterized by active lesions. The third ROC curve for both categories was made wherein only M2 was considered positive and all other M stages were considered negative (Method 3). All missing data were excluded and M4.1 lesions were allocated under M1 lesions throughout the whole study.

Objective 2: Determine if different stages of DD lesions can be distinguished using IRT images

This objective concerns the question whether or not IRT is able to make more precise distinctions between the different DD lesions. Therefore, the lesions stages were subdivided in three categories: absence of DD (M0), active DD lesions (M1/M2) and chronic lesions of DD (M3/M4). Then, the mean maximum temperature of each of categories was calculated and compared to the other ones using independent t-tests. This was done for both pre- and post-images and there was no distinction between data from the left and the right foot.

Objective 3: Determine if lameness is related to inflammation in the hoof as measured by IRT

Six ROC curves were produced for the temperature of the hottest foot, as measured by IRT, compared to the lameness stage, to determine if lameness is related to inflammation in the hoof. The distinction between pre- and post-hosing of the legs was maintained: three pre-hosing ROC curves and three post-hosing ROC curves were made. The three ROC curves in both categories were produced for different 'negative' and 'positive' events: lameness score 1 was considered negative and lameness scores 2, 3, 4 and 5 were considered positive (1), lameness score 1 and 2 were considered negative and lameness score 3, 4 and 5 were considered positive (2) and lameness score 1, 2, 3 were considered negative and lameness score 4 and 5 were considered positive (3).

Objective 4: Determine if the use of a cost-friendly camera would obtain the same results as a more expensive camera

To determine the correlation between the two different cameras, the correlation coefficient (r) was calculated using the Excel 2013 correlation formula and a scatterplot. The correlation coefficient uses two sets of continuous data of the same observations and calculates whether or not they are comparable. In other words, the degree of association is measured by calculating Pearson's product moment correlation coefficient (Petrie & Watson, 2013). The correlation coefficient can take any value from -1 to +1, whereas -1 is considered the perfect negative correlation and +1 the perfect positive correlation. The closer the value of r to either one of the extremes, the stronger the correlation between the two variables (Petrie & Watson, 2013; Schober et al., 2018). The Excel correlation formula was used to perform this calculation, for three separate comparisons between the two cameras: all pre-hosing left and pre-hosing right feet taken together (1), all post-hosing left and post-hosing right feet taken together (2) and all pre- and post-hosing left and pre- and post-hosing right feet taken together (3). In addition, scatterplots with a trend line and its formulas were produced, together with the function to show r^2 which is the square of the correlation coefficient (Petrie & Watson, 2013). This was used as a tool to double-check the outcome of r provided by the correlation formula.

Objective 5: Determine the difference between IRT images made before and after hosing of the hooves

The correlation coefficient was used to determine if IRT images that were made before hosing were correlated to IRT images that were made after hosing, in the same way as the two cameras were compared. Two correlations were determined: pre- versus post-hosing for the left and right legs together using camera I (1) and pre- versus post-hosing for the left and right legs together using camera II (2). Scatterplots were made to confirm the r-values that were received from the correlation formula.

RESULTS

In total, there were 1404 pre-hosing images and 1479 post-hosing images of good quality from camera I and 219 pre-hosing images and 212 post-hosing images of good quality from camera II, which could be further divided in the different M stages.

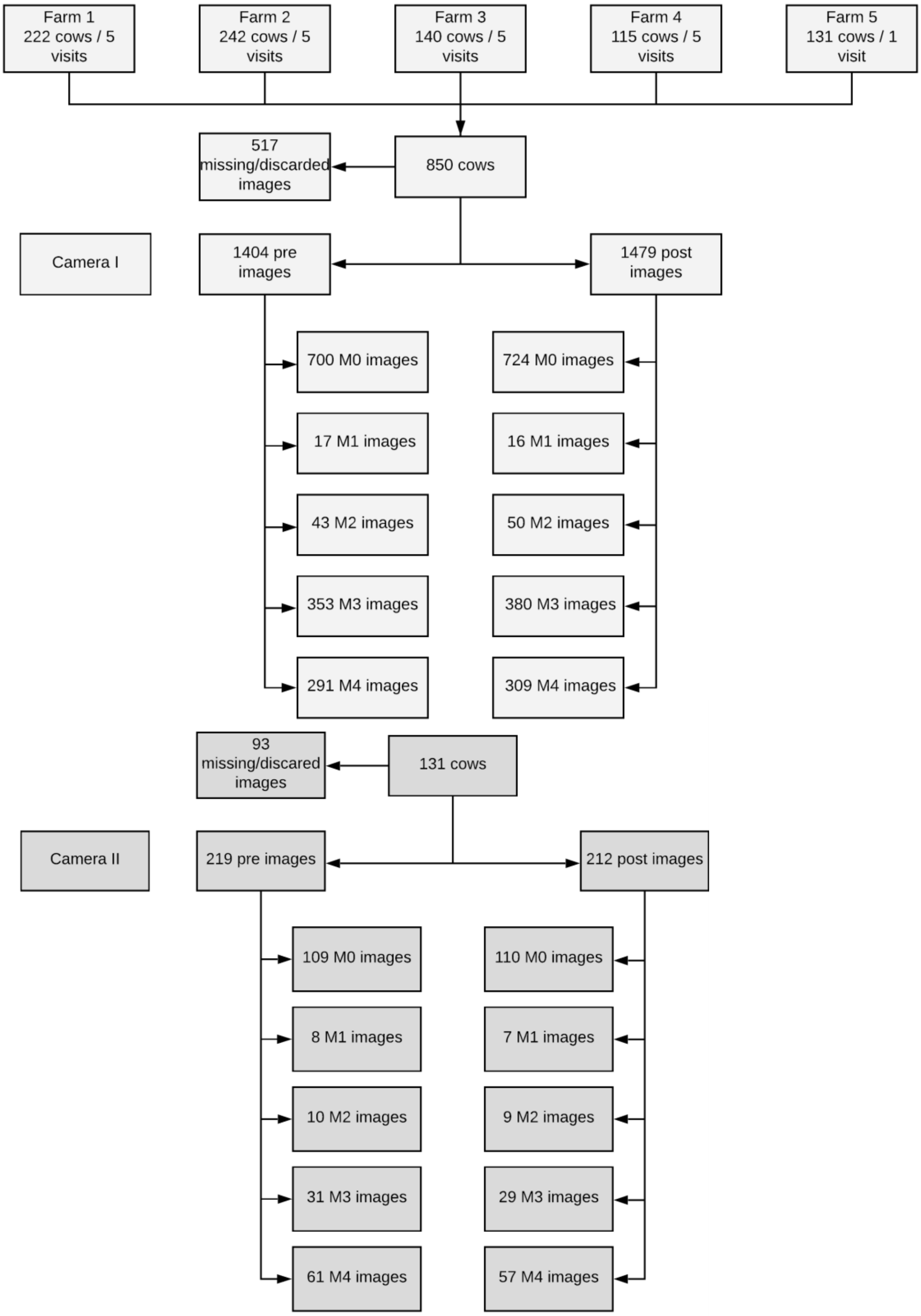

Figure 6: Flowchart with an overview of the total number of images, divided in 'pre images' and 'post images' reflecting images taken before hosing of the feet and after hosing of the feet respectively.

Objective 1: Determine if IRT can be used to detect DD lesions that are visibly identifiable

The results of the t-tests are listed in Table 2 A, B and C. The p-values of the t-tests for pre- and post-hosing for both M0 versus M2 and for M2 versus all of the other DD stages didn't exceed the 0.05 p-value and can therefore be considered as significant. Also the t-test for the difference in the mean of the temperatures of M0 versus all other M stages was significant for the pre-hosing images, but not for the post-hosing images.

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (M0 vs M2)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
M0	31.1	31.2	30.9 – 31.3	31.0 – 31.4	0.007	0.027
M2	32.2	32.0	31.8 – 32.5	31.6 – 32.5		

Table 2A: Mean maximum temperature, 95% confidence interval and p-value the of t-test for M0 versus M2.

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (M2 vs M0/M1/M3/M4)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
M2	32.2	32.0	31.8 – 32.5	31.6 – 32.5	0.013	0.030
M0/M1/M3/M4	31.3	31.3	31.3 – 31.4	31.2 – 31.4		

Table 2B: Mean maximum temperature, 95% confidence interval and p-value the of t-test for M2 versus all other DD stages.

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (M0 vs M1/M2/M3/M4)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
M0	31.1	31.2	30.9 – 31.3	31.0 – 31.4	0.005	0.080
M1/M2/M3/M4	31.5	31.4	31.3 – 31.6	31.3 – 31.6		

Table 2C: Mean maximum temperature, 95% confidence interval and p-value the of t-test for M0 versus all other DD stages.

The number of the ROC curve, the DD stages that were considered positive and negative, the AUC's, the Cut-off temperatures and the sensitivities and specificities for the six ROC curves are shown in Table 3. All of the AUC values lie within the 0.508 – 0.615 range. Figure 7 shows the ROC curve with the highest AUC, which is ROC curve 3.

ROC	Pre/Post	Positive	Negative	AUC	Cut-Off Temp. (°C)	Se. – Sp.
1	Pre-hosing	M1/M2/M3/M4	M0	0.533	31.1	0.642 – 0.419
2	Pre-hosing	M1/M2	M0/M3/M4	0.600	31.2	0.768 – 0.415
3	Pre-hosing	M2	M0/M1/M3/M4	0.615	31.2	0.844 – 0.414
4	Post-hosing	M1/M2/M3/M4	M0	0.508	29.5	0.836 – 0.215
5	Post-hosing	M1/M2	M0/M3/M4	0.576	31.1	0.763 – 0.392
6	Post-hosing	M2	M0/M1/M3/M4	0.583	31.1	0.811 – 0.391

Table 3: Survey of all different ROC curves for both feet, pre- and post-hosing. 'Positive' and 'Negative' represent the M stages that were chosen to distinguish. AUC represents the Area Under the Curve, the dark grey line in Figure 7. The Cut-Off Temp. represents the matching cut-off temperature for each AUC. 'Se. – Sp.' represents the sensitivity and the specificity of the cut-off temperature respectively with 0 as minimum and 1 as maximum.

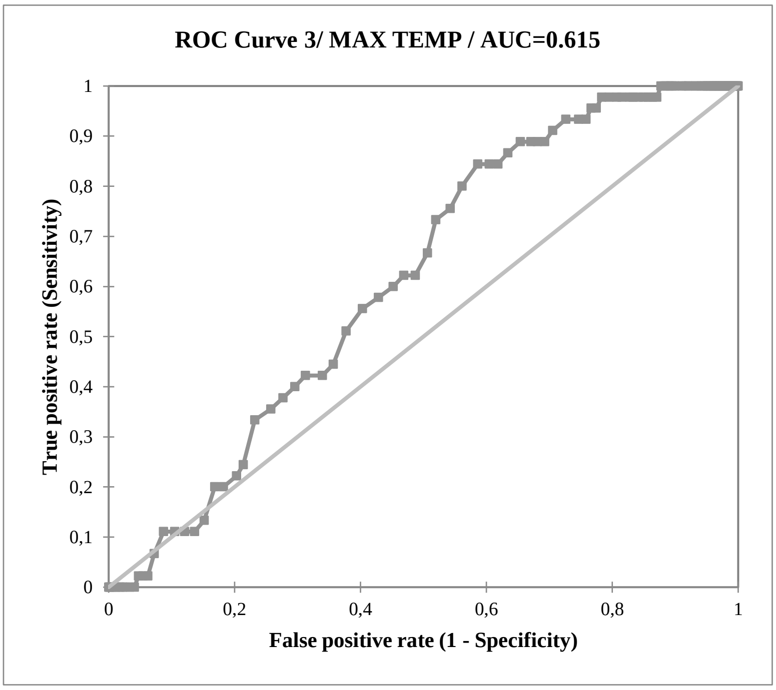

Figure 7: ROC Curve with an AUC of 0.615 and a cut-off value of 31.2 °C, with a sensitivity of 0.844 and a specificity of 0.414.

Objective 2: Determine if different stages of DD lesions can be distinguished using IRT images

The results of the three independent t-tests are listed in Table 4A, B and C. The p-values for each of the three t-tests with pre-images didn't exceed 0.05 and the matching categories are therefore significantly different. This applies for the t-test between 'absence' and 'active' with post-images as well, which has a p-value of 0.046. The two remaining t-tests for post-images resulted in p-values of 0.078 and 0.172 and are therefore not significant.

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (absence vs active)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
Absence	31.1	31.2	30.9 – 31.3	31.0 – 31.4	0.003	0.046
Active	32.1	31.9	31.7 – 32.4	31.4 – 32.3		

Table 4A: Mean maximum temperature, 95% confidence interval and p-value of the t-test for absence (M0) of DD versus active DD lesions (M1/M2)

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (absence vs chronic)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
Absence	31.1	31.2	30.9 – 31.3	31.0 – 31.4	0.022	0.078
Chronic	31.4	31.4	31.2 – 31.6	31.2 – 31.5		

Table 4B: Mean maximum temperature, 95% confidence interval and p-value of the t-test for absence (M0) of DD versus chronic DD lesions (M3/M))

	Mean temp. (°C)		**95 % Confidence Interval (°C)**		**P-value (active vs chronic)**	
	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing	Pre-hosing	Post-hosing
Active	32.1	31.9	31.7 – 32.4	31.4 – 32.3	0.017	0.172
Chronic	31.4	31.4	31.2 – 31.6	31.2 – 31.5		

Table 4C: Mean maximum temperature, 95% confidence interval and p-value of the t-test for active DD lesions (M1/M2) versus chronic DD lesions (M3/M4)

Objective 3: Determine if lameness is related to inflammation in the hoof as measured by IRT

Table 5 shows an overview of the six different ROC curves. All the AUC values are within a small range, from 0.478 to 0.610, around the 'chance-value' of 0.5. The ROC curve with the highest AUC (ROC 6) is found in Figure 8.

ROC	Pre/Post	Positive	Negative	AUC	Cut-Off Temp. (°C)	Se. – Sp.
1	Pre-hosing	2, 3, 4, 5	1	0.508	30.5	0.893 – 0.181
2	Pre-hosing	3, 4, 5	1, 2	0.509	30.7	0.955 – 0.174
3	Pre-hosing	4, 5	1, 2, 3	0.478	31.2	0.895 – 0.245
4	Post-hosing	2, 3, 4, 5	1	0.487	27.9	0.978 – 0.070
5	Post-hosing	3, 4, 5	1, 2	0.531	32.2	0.632 – 0.466
6	Post-hosing	4, 5	1, 2, 3	0.610	33.2	0.500 – 0.697

Table 5: Survey of all different ROC curves for different lameness scores. The number in the column 'Positive' and 'Negative' represent the lameness scores that were chosen to distinguish. AUC represents the Area Under the Curve, the dark grey line in Figure 8. The Cut-Off Temp. represents the matching cut-off temperature for each AUC. 'Se. – Sp.' represents the sensitivity and the specificity of the cut-off temperature respectively with 0 as minimum and 1 as maximum.

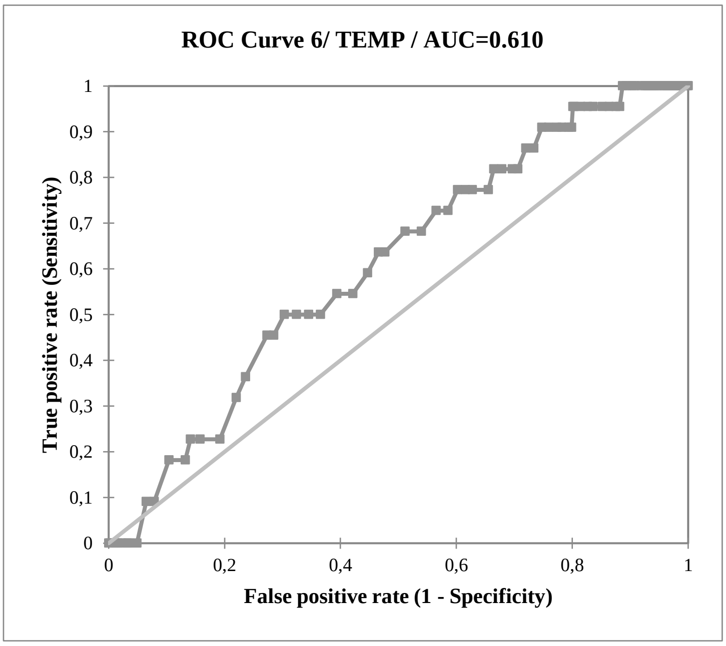

Figure 8: ROC Curve with an AUC of 0.610 and a cut-off value of 33.2 °C with a sensitivity of 0.50 and a specificity of 0.697.

Objective 4: Determine if the use of a cost-friendly camera would obtain the same results as a more expensive camera

The correlation coefficients between the two cameras were 0.939, 0.925 and 0.932 respectively. (Table 6). An r-value that exceeds 0.90 is considered a very strong correlation (Schober et al., 2018), which applies for all r-values of this objective. Figure 9 shows the scatterplot for the comparison with the highest r (comparison 1): camera I compared to camera II for all of the pre-hosing images together. The formula for the trend line is showed, as well as the r^2. By taking the square root of r^2.

Comparison no.	Camera I	Camera II	r
1	All pre-hosing feet	All pre-hosing feet	0.939
2	All post-hosing feet	All post-hosing feet	0.925
3	All pre- and post-hosing feet	All pre- and post-hosing feet	0.932

Table 6: The r for three different comparisons between camera I and II. 'All pre-hosing feet' and 'all post-hosing feet' reflect the pre-hosing left and pre-hosing right feet taken together and the post-hosing left and the post-hosing right feet taken together, respectively.

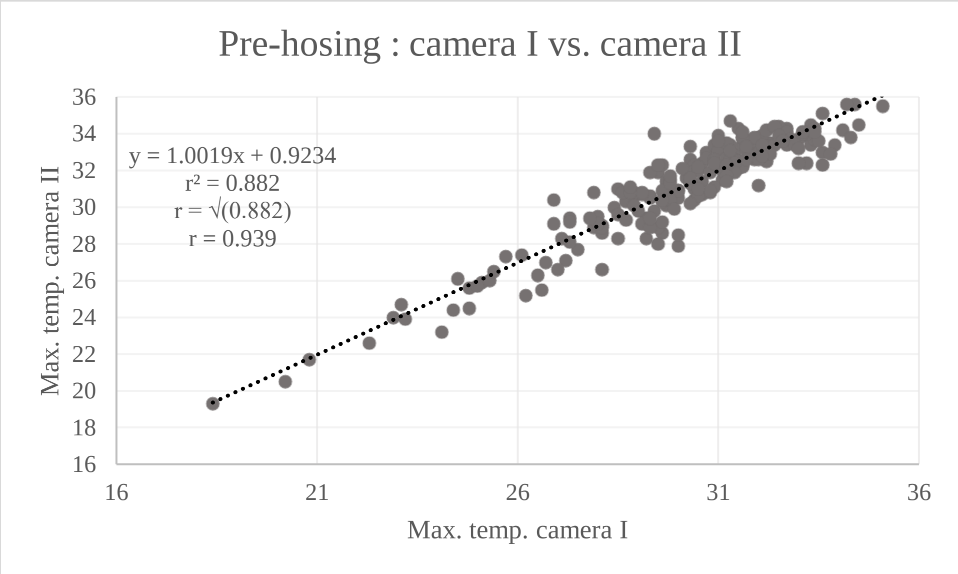

Figure 9: scatterplot of comparison one. 'Max. temp. camera I' and 'Max. temp. camera II' stand for the maximum temperature that was measured with camera I and camera II, respectively.

Objective 5: Determine the difference between IRT images made before and after hosing of the hooves

The correlation coefficients for both categories described in the materials and methods are shown in Table 7. Both comparisons between pre- and post-hosing of the legs result in r-values that exceed 0.70 and can therefore be considered as strong correlations (Schober et al., 2018). The highest correlation is found in the results of camera II. The graph of the comparison with the highest r (comparison 2) is shown in Figure 10.

Comparison no.	Pre-hosing	Post-hosing	r
1	Camera I	Camera I	0.733
2	Camera II	Camera II	0.817

Table 7: The r value for two different comparisons between pre- and post-hosing of the hooves. The two cameras are represented and the images of the left and right legs have been taken together.

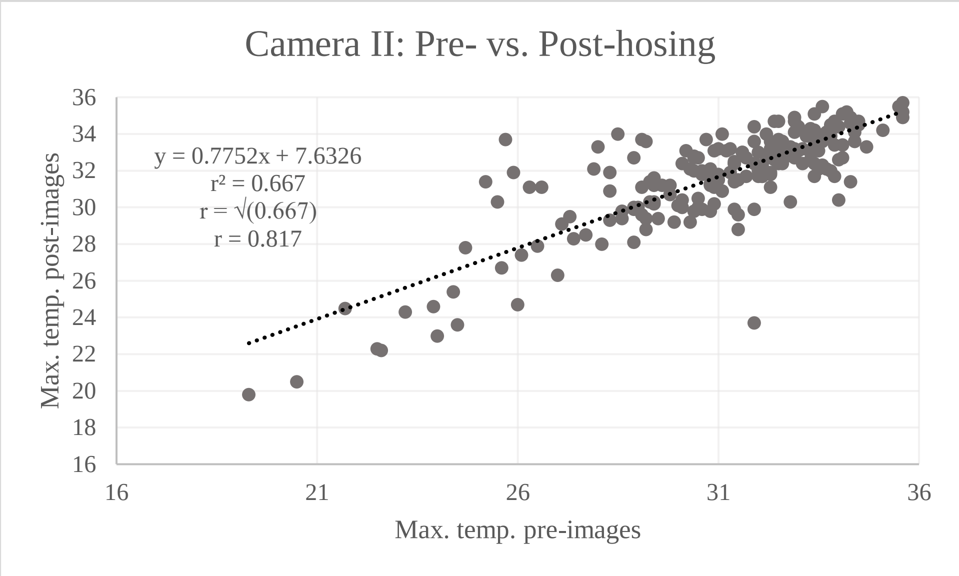

Figure 10: Scatterplot of comparison two. 'Max. temp. pre images' and 'Max. temp. post images' represent the maximum temperature of pre- and post-hosing images of the legs, respectively.

DISCUSSION

The use of IRT in lameness and hoof lesion detection has been investigated in several studies (Alsaaod & Buscher, 2012), but little is known about DD detection specifically. The fact that it is a non-invasive method which could be applied any time without having to wait for the next trimming round, makes it a promising new method of DD detection. This study showed that there are significant differences between the temperatures of feet with DD and feet without DD. Also it showed that the three main categories of 'absence of DD', 'active DD lesions' and 'chronic DD lesions' were significantly different for images taken without hosing of the feet. For the post-images only the difference between 'absence' and 'active DD lesions' was statistically significant. This is interesting regarding a possible on-farm implementation, where it would be beneficial if hosing isn't necessary to detect DD.

However, the AUC values of the ROC curves for the different DD stages are less convincing as they all lie within the 0.508 and 0.615 range, which is close to the chance value of 0.5. The 0.615 AUC value was found in pre-hosing data wherein only M2 was considered a positive event. This happens to be the most interesting category considering the pre-hosing data as most relevant regarding a possible on-farm implementation and considering M2 the most relevant lesion regarding spread of disease and treatment. The sensitivity of 0.844 indicates that 84.4% of cows with M2 lesions will be detected which is a considerable high proportion. On the other side, the specificity of 0.414 indicates that only 41.4 % of the cows not having M2 lesions will be identified so. This means that 58.6 % of the cows not having M2 lesions will be identified as having M2 lesions and are therefore false positive (Petrie & Watson, 2013) which wouldn't be useful in on-farm implementation. This corresponds with data from Alsaaod and Büscher (2012) who investigated the use of IRT in detecting hoof lesions in general and made a distinction between before and after hoof trimming. They found an AUC of 0.689 for both the before and after hoof trimming ROC curves. A 2014 study from the same authors focused on IRT in detecting DD specifically and made a distinction between two regions: the coronary band and the skin above that. In addition, they compared temperatures of rear feet to front feet to detect DD, which resulted in an ROC with an AUC of 0.842, a sensitivity of 0.891 and a specificity of 0.666 (Alsaaod et al., 2014). However, sensitivity and specificity were lower when both front and rear feet were affected: 0.600 and 0.625 respectively. Also Stokes et al. (2012) investigated the use of IRT in detecting hoof lesions, and made a distinction between dirty and clean feet, but added a category: lifted feet. For all of the categories images were taken in a similar way to this study, from the back side of the hind feet focusing on the pastern. They found sensitivities of 0.80, 0.91, and 0.93 respectively with specificities of 0.73, 0.54 and 0.49 (Stokes et al., 2012a), which corresponds with the sensitivity and specificity of the highest AUC in this study. They concluded that they found an association between hoof lesions, including DD, and an elevated hoof temperature, but that they were not able to distinguish between different lesions (Stokes et al., 2012a). However, for on-farm implementation in the future, higher specificities are needed for a more reliable distinction between cows having DD and cows not having DD. A lower specificity results in a misclassification of cows not having DD but being classified as having DD. In other words, the true negative rate decreases. Therefore more research is needed before IRT could be implemented on-farm, but the fact that there are significant differences in temperature between DD stages is promising for research in the future.

Remaining findings

Objective 3: Determine if lameness is related to inflammation in the hoof as measured by IRT

The AUC's from all of the different ROC's approach 0.5, which indicates that the use of IRT in detecting lameness is close to detecting lameness by chance. This can be explained by the fact that lameness can occur in both front and hind legs (Cramer et al., 2008) and that only the hind legs were used for obtaining data in this study. This means that every cow that was lame due to a cause located in either one or both of the front legs or upper legs, was judged by the temperature of the hind legs. In addition, for each cows only the hottest foot was taken into account, which means that the other foot was ignored whether or not is was affected by DD or another hoof lesion.

Objective 4: Determine if the use of a cost-friendly camera would obtain the same results as a more expensive camera

The correlation coefficient of all seven comparisons between the two different cameras was higher than 0.90 and can therefore be considered as 'very strong' (Schober et al., 2018). The highest agreement was found in the comparison of the two cameras of the left leg before hosing. This indicates that the difference between the two cameras is very small which might be relevant for on-farm implementation of IRT cameras, as the purchase of a cost-friendly camera might be more feasible for producers. However, for possible other implementations of IRT, it must be taken in consideration that camera II differed from camera I in the software. Therefore it might lead to different results when it is used for other purposes than obtaining the maximum temperature, as was done in this research.

Objective 5: Determine the difference between IRT images made before and after hosing of the hooves

The correlation coefficient exceeded 0.70 for all of the six comparisons between before and after hosing off the feet and can therefore be considered as 'strong' (Schober et al., 2018). This might be relevant regarding the need for hosing of the hooves before scoring DD. Up to now, hosing of feet is highly recommended when scoring of DD is done in the milking parlour, but producers can be reluctant to do so due to reasons of udder contamination and extra labour (Oliveira et al., 2017). Therefore, IRT could be implemented on farm for detecting DD as washing of the hooves is not necessary when using IRT.

Limitations of the study

One important limitation of the study regarding the IRT software was that analysing of the images was done manually by drawing a box, as described in the materials and methods, and can therefore be considered as a skill which isn't objective. In addition, analysing the IRT images one by one was time consuming. The box was drawn for each image individually, which might lead to differences in the position of the box and thus to different results. However, to minimise this risk, all of the images were analysed using one procedure for drawing the box (for description see materials and methods) to be sure that the analysis was done the same way for each image. Also, to avoid interobserver disagreement all of the images were analysed by one person.

Another limitation of using the box for analysing a part of the leg, is that sometimes DD lesions can occur outside the region of the drawn box. These lesions are visually scored as DD lesions, but there is a chance that they might not reflect a higher temperature in the box, as the box doesn't include them. In addition, the opposite might be the case as well: DD lesions can occur where they are not always visible (Solano et al., 2017). When that is the case, the hooves would have been classified as 'healthy', whereas they actually are affected with DD. Even though the lesion is not visible, the temperature of the foot could be elevated anyways due to the lesion. This results in a false M0 score but in a higher temperature. In addition, there is a chance that cows are developing lesions, which are not visible yet. As a result, they might have an elevated hoof temperature, but cannot be classified as having DD.

There are certain limitations considering the part of detecting and scoring of the DD lesions. At first, there is the possibility that not all of the lesions were detected and properly classified, as the feet were scored in the milking parlour. Even though this is a reliable way to determine DD prevalence on herd-level, it cannot replace the trimming chute as a way of detecting DD (Solano et al., 2017). Second, all of the M4.1 stages have been classified under the M1 stage.

Several limitations go with the data collection part. First of all, all of the farms were visited in the period of May to August, except for the one farm that was visited only once. That farm was visited in November, when ambient temperatures are remarkably lower than in summer (Government of Canada, 2018). Although the IRT cameras were calibrated for the ambient temperature at the beginning of each visit, there is a possibility that the environmental temperature might have had an influence on body temperature of the cows as the body temperatures increases with increasing ambient temperatures (Alsaaod & Buscher, 2012). Same limitation is the case for the timing of each visit as there was a difference in the milking times that were attended. Each farm was only visited during the same milking round, but there was variation among the farms. For example one farm was always visited during the morning milking round and another farm has always been visited during the afternoon milking round. As the body temperature of cows shows a certain circadian rhythm (Kendall, 2009), this might have affected the results of the measurements by the one IRT camera, but not for the comparison between the two cameras as only the farm which was visited once was used for that objective. Similar to changes in body temperature during the day, a cows body temperature can also be raised after a moment of stress (Stewart et al., 2007), which can be the case when the feet are getting hosed for example. However, a 2012 study from Stokes et al. reflecting on the use of IRT in foot lesion detection, showed that calibrating for skin temperature was not necessary.

Another limitation is that the time between the pre-hosing image and the post-hosing image is not constant. The amount of variation in time between the two groups of images is unclear. This might lead to affected results, as the hosing of the feet may have an impact on the temperature of the hoof. The exact impact is unsure at is has been described that debris on the hooves might work as an insulator and that dirty hooves therefore reflect a lower temperature than they actually are (Van Hoogmoed et al., 2000). On the other side, hosing can also have a cooling effect on the hooves. When the post-hosing image is taken after a longer period, the legs and the hooves have more time to dry which might lead to higher temperatures of the feet.

This goes for the comparison between the two cameras as well. There is a certain variation in time between the images of the first camera and when the images with the second camera were taken. The bigger the difference in time between these, the greater the chance that the cow moves one of her feet. An image from a foot in a different position might result in different outcomes. These differences in outcome cannot be related to the difference in camera.

Consistent with other papers on the use of IRT in lameness in cattle, the variable 'maximum temperature of the hoof' was used for the calculations for each objective, as it is believed that the maximum temperature reflects lesions more consistent than minimum temperature (Stokes et al., 2012a). However, it must be taken in consideration that these studies investigated the use of IRT in lameness or hoof lesions in general and didn't particularly focus on DD.

CONCLUSION

This study showed that IRT was able to make a significant distinction between absence of DD and active DD lesions, between active DD lesions and chronic DD lesions and between absence of DD and chronic DD lesions, but lacked a high combination of sensitivity and specificity. The results obtained from the pre- and post-hosing images were strongly correlated, which shows potential for on-farm implementation. Also there is only a very small difference in results obtained from the expensive camera compared to those of the cost-friendly camera which pleads for a possible use on-farm of a cost-friendly camera. Infrared thermography is a promising new method of detecting DD in dairy cows but more research is needed before on-farm implementation for detection and early treatment can be recommended.

ACKNOWLEDGEMENTS

This research would never have been possible without the financial contribution of Alberta Milk (Edmonton, Alberta, Canada) who provided funding for this research as well as the willingness of its members to participate in this study. In addition I would like to thank Dr. Karin Orsel and all members of the Orsel team (Department of Production Animal Health, University of Calgary, AB, Canada) for the supervision, Casey Jacobs (University of Calgary, AB, Canada) for the data collection and help with the statistical analysis, as well as Kelsey Gray (University of Calgary, AB, Canada) for the data collection.

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