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Infrared thermography of the udder surface of dairy cattle: Characteristics, methods, and correlation with rectal temperature

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ABSTRACT

Thermograms of the caudal udder surface were taken of five healthy cows before and after inoculation of *Escherichia coli* into the right hind quarter. Images in clinically normal udder quarters from cows without fever (CN) were compared with those post inoculation when cows had fever ($\geqslant$39.5 °C) and showed elevation of somatic cell counts ($\geqslant$400,000 cells/mL) in the inoculated quarter (CM). Using graphic software tools, different geometric analysis tools (GATs: polygons, rectangles, lines) were set within the thermographic images. The following descriptive parameters (DPs) were employed: minimum value ('min'), maximum value ('max'), range ('max–min'), and arithmetic mean ('am').

Surface temperatures in group CN were between 34.1 °C ('polygons'/'min') and 37.9 °C ('polygons'/'max'), and in group CM between 34.5 °C ('polygons'/'min') and 40.0 °C ('polygons'/'max'). The greatest differences in the temperatures between CN and CM (2.06 °C) were found in 'polygons' and 'rectangles' using 'max'. The smallest coefficient of variation in triplicate determinations was found in GAT 'polygons' with DP 'max' (T_{max}) (0.15%), and the relationship to the rectal body temperature (T_r) could be described by $T_r = 5.68 + 0.874 * T_{max}$. The results show that significant changes can be displayed best using the GAT 'polygons' and the DP 'max'. These methods should be considered for automated monitoring of udder health in dairy cows.

Introduction

Infrared thermography (IRT) is a non-invasive diagnostic tool for visualizing and analysing local and temporal changes in surface temperatures. The result is a thermogram, in which each pixel relates to a temperature value. Humans and most other mammals produce excess body heat which has to be lost to the environment. An important regulator in this interplay is the microcirculation of the skin (Robinson, 1992) and IRT could be of diagnostic value in inflammatory processes that are accompanied by changes in peripheral blood circulation, and so with local or general heat balance.

Mastitis has negative effects on milk production and quality and is a significant economic problem (Bar et al., 2008) that requires considerable farm staff time in detecting. Automated early detection of changes in udder health could offer cost efficiencies. As the surface temperature of the skin reflects the underlying blood circulation and the metabolism of the tissue (Paulrud et al., 2002), some types of mastitis may cause an increase in the skin temperature of the udder (Colak et al., 2008; Polat et al., 2010), especially if mastitis is accompanied by fever (Hovinen et al., 2008).

There have been only a few detailed studies of IRT as a method for diagnosing mastitis (Paulrud et al., 2002; Pezeshki et al., 2011), mainly due to the high technical and financial input required even for minimal standards of quantification. Obtaining reliable IR data from a live animal is challenging and to date temperature patterns required interpretation by trained persons (Jiang et al., 2005). Computer-aided analysis can enhance reliability and objectivity (Ng and Fok, 2000; Jiang et al., 2005) but, so far, no studies of standardized IRT of the udder surface have been reported. The primary objective of the present study was to compare different algorithms for the evaluation of udder skin thermography pictures with the aim of obtaining objective and valid results for future automated computer-supported processing and detection of acute mastitis and fever.

Materials and methods

The study was approved by the Animal Ethics Committee of the regional government of Upper Bavaria (reference: 55.2-1-54-2531-108-05). Five first lactation Holstein–Friesian cows were used ranging from 2 to 6 months post calving and with a daily milk yield of 20–30 L. Prior to the study, the udders of all five cows had been examined and determined to be healthy; samples from all quarters from each cow tested negative for bacteria, and individual quarter samples had been shown to have somatic cell counts (SCC) of <50,000 cells/mL (determined with Fossomatic-5000, FOSS).

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Twenty-four hours after the start of the study, 2 mL of *Escherichia coli* suspension (250 colony-forming units in physiological saline/mL) were instilled into the teat cistern of the right hind quarter of each cow. As a placebo treatment, 2 mL of sterile physiological saline were instilled into the left hind quarter of the same animal. Additional milk samples for determination of SCC and bacteriological culture were taken from all quarters just prior to inoculation, and 12 and 24 h later.

Thermograms of the caudal aspects of the udder were taken from a distance of 1.80 m (in a straight line from behind the animal). A series of thermograms was taken of the hind quarters starting 24 h before until 24 h after inoculation. Thermograms were taken every 2 h from 05:30 to 17:30 h, and additionally at 08:30, 06:30, and 18:00 h (the latter two were just after milking), thus giving a total of 16 time points per day. Inoculation of *E. coli* was undertaken after morning milking at 06:30 h.

For recording, a B20 HSV infrared camera (FLIR Systems, image frequency 50/60 Hz, spectral band 7.5–13 μm, 640 × 480 pixel, non-cooled sensor, thermal sensitivity <0.05 °C at 30 °C, standard 25 Hz, precision of the imaging ± 2% of the recorded value) was used. Emissivity value was set at $\varepsilon = 0.96$. A digital ultrasound device was used to measure the exact distance between camera objective and udder skin plus a data logger to measure the environmental temperature and humidity and a digital thermometer to measure the rectal temperature. Prior to all measurements the hair of the udder skin was clipped. The skin of the examined udder quarters was cleaned if judged necessary (dry or slightly wet, using only water), but in order to avoid artefacts this was done at least 10 min before measurements. For a standardized analysis, the upper image borderline was marked on the udder at the height of the stifle joint with an adhesive tape.

Geometric analysis tools (GATs)

A Thermocam-Researcher 2.8 Pro Version Nero (FLIR Systems) was used and each image was analysed individually. Of a series of five infrared images per measurement, the first two images were discarded, as the camera needed one to two images in order to focus exactly, and three images per measurement were analysed. In addition, a correction for ambient temperature and humidity was made by introducing these parameters into the analytical software. In order to avoid the influence of subjectivity on the interpretation of the images, analysis was done on a personal computer using three different geometric software-tools: 'polygons', 'rectangles' and 'lines'.

Polygons

The individual udder quarters were measured using manual tracing, excluding the teat, applying the polygon-tool of the software (see Fig. 1, dashed line).

Rectangles

In each of the quarters, a standardized rectangle in relation to the size of the individual quarter was inserted: the height of the rectangle was 75% of the distance between the base of the teat to the tape, starting at the distal point of the intermammary groove; the width of the rectangle was 50% of the width of the udder quarter at the height of the lower end of the intermammary groove (see Fig. 1, solid line).

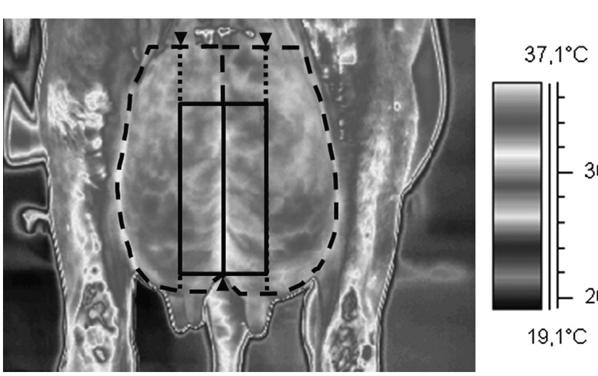

Fig. 1. Definition of analysis tools for the analysis of thermographic images of the udder skin of dairy cows. Polygons: dashed line (using the intermammary groove and the contour of the udder). Rectangular areas: solid line (the height of the rectangle was 75% of the distance between the base of the teat to the tape, starting at the distal point of the intermammary groove (▲); the width of the rectangle was 50% of the width of the udder quarter at the height of the lower end of the intermammary groove). Lines: dotted line (straight lines were drawn on the outer borders of the rectangles starting at the bottom line of the tape mark (▼) and ending at the base of the teat).

Lines

Using the rectangles of the second tool, straight lines were drawn on the outer borders of the rectangles starting at the bottom line of the tape mark and ending at the base of the teat (see Fig. 1, dotted line).

Descriptive parameters (DPs)

At each time point, three images were analysed and three values for each of the following DPs were obtained: minimum ('min'), maximum ('max'), difference between maximum and minimum ('max–min'), arithmetic mean temperature ('am') and standard deviation (SD). For each of the DPs, the arithmetic mean of the three values was determined.

Grouping

To determine the precision of measurements in clinically healthy cows and in cows with clinical mastitis, the average coefficients of variation (CV) were calculated for the 'min'-, 'max'-, 'max–min'- and the 'am'-values of three consecutive thermograms. In five cows, a total of 160 series of three images of left and right hindquarters taken at 16 time points prior to inoculation, was analysed (clinically normal udder quarters, group CN). To evaluate GATs and DPs in clinical mastitis cases, only measurements at the inoculated right hindquarters were used at those time points after inoculation, when the animals had a rectal temperature of ⩾39.5 °C and elevated somatic cell counts (⩾400,000 cells/mL) (clinical mastitis, group CM).

To determine the association between rectal temperature and thermographically determined surface temperature, arithmetic means of the series of three consecutive measurements of all 32 time points in five animals were used, stratified for both hind quarters.

Calculations and subsequent analyses were conducted using SPSS 19.0 (IBM). The Kruskal–Wallis test for independent samples was used with subsequent Bonferroni-corrected Mann–Whitney tests for pair-wise comparisons. Additionally, a mixed model was conducted with SAS 9.2 (SAS Institute), taking repeated measurements into account. Fixed effects included time and quarter, with animal as a random effect. For determination of the optimum cut-off point for identifying animals with rectal temperatures of ⩾39.5 °C and SCC ⩾ 400,000 cells/mL, a ROC analysis was conducted for the different geometric analysis tools using the different descriptive parameters. The optimum cut-off point was defined as the maximum of the sum of sensitivity and specificity. Associations between rectal temperatures and surface temperatures were analysed using linear regression.

Results

Prior to inoculation with *E. coli*, all animals had rectal temperatures <39.5 °C and SCC < 50,000 cells/mL in all quarters at all time points. Following inoculation, rectal temperatures of ⩾39.5 °C were detected twice in one cow, three times in two animals, four times in one animal, and five times in one animal, all of them with SCC ⩾ 400,000 cells/mL and *E. coli* positive in bacteriological culture of quarter milk samples in the inoculated quarter 12 h after inoculation, thus providing 17 measurements in this group (CM).

Medians of thermographically determined surface temperatures of the udder ranged from 34.1 °C ('polygons'/'min') to 37.7 °C ('polygons'/'max') in group CN and from 34.5 °C ('polygons'/'min') to 39.7 °C ('polygons'/'max') in group CM (Table 1). Before experimental inoculation, there were no significant differences in udder surface temperatures between left and right hind quarters with all three GATs and in all three DPs. In group CM, significantly higher temperatures were found with all three GATs for all DPs, except for 'min'. The greatest differences in median temperatures between CM and CN were found in the GAT 'polygons' and 'rectangles', using 'max', where the difference in both GATs was 2.06 °C.

The greatest interquartile range was found for the DP of 'min' in all three GATs and in both CN and CM. The standard deviation was lowest in the GAT 'lines' in group CN, and was significantly higher for all GATs in group CM compared to group CN (Table 1). Statistically significant differences in the SD between the GATs were found in group CN between 'polygons' and 'lines' ($P < 0.001$) and between 'rectangles' and 'lines' ($P < 0.001$) whereas SD was not statistically different between 'polygons' and 'rectangles'. No