Infrared thermography to detect intramammary infections at drying off

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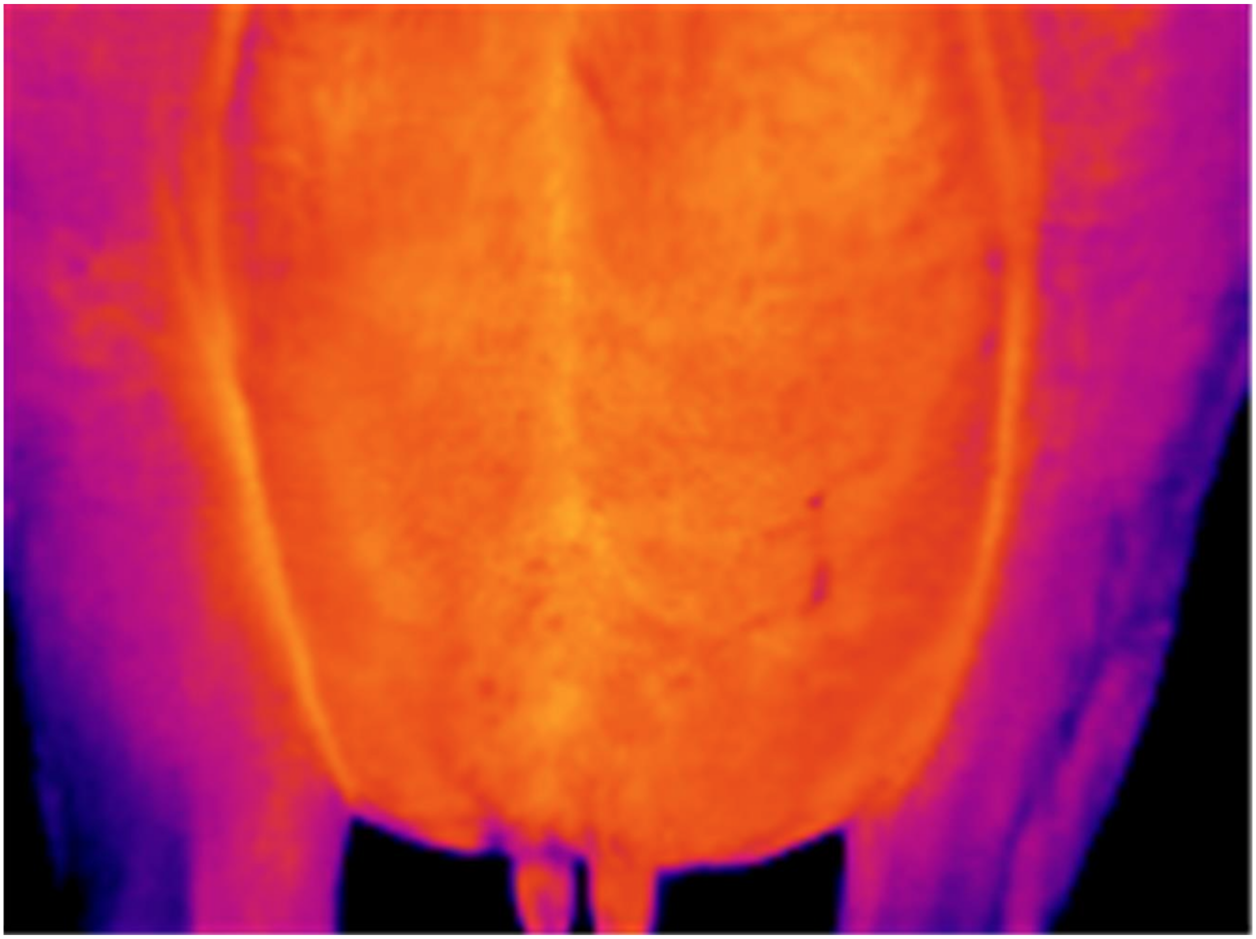

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Abstract

Mastitis is one of the most common problems in the dairy industry and is responsible for large economic losses. Mastitis is mainly caused by bacterial intramammary infections (IMI). During the dry period, especially at the start and the end of that period, mammary glands are susceptible to the invasion of udder pathogens. IMI gained during the dry period is a risk factor for the development of clinical mastitis after calving. Antibiotic treatment at drying off has a dual target: cure existing IMI and prevention of new IMI during the dry period. Currently, decisions about the dry off treatment are usually based on cow level diagnostics. To optimize antimicrobial use in dry cow treatments, a next step may be to make dry cow treatment decisions at quarter level rather than on cow level. Infrared thermography (IRT) is a simple, effective and non-invasive method that detects surface heat. In experimental settings it has been shown to be effective in diagnosing clinical mastitis. To determine if IRT is able to diagnose subclinical mastitis at drying off, a total of 53 dairy cows from 3 different dairy farms have been photographed. Somatic cell count (SCC) on cow level, on quarter level and a bacterial culture at quarter level were used to determine if IRT could be useful as a quarter level diagnostic on top of SCC on cow level to diagnose subclinical mastitis.

The mean quarter temperature measured at a distance of 1 meter varied between 26℃ and 36.8℃. The maximum quarter temperature varied between 29.2℃ and 43.3. There is no significant association between the SCC on quarter level and the mean and maximum quarter temperature. No significant association was found between the presence of an IMI and the mean and maximum quarter temperature. Quarters with a major pathogen IMI, which could benefit from antibiotic treatment at drying off, were considered to have subclinical mastitis. No association was found between the presence of subclinical mastitis and the mean and maximum quarter temperature.

The IRT pictures were taken the day after drying off. To check if a difference in quarter temperature occurred between the day of drying off and the day after, a total of 13 dairy cows were photographed using the IRT camera. There is a significant association between the quarter temperature measured at day 1 or day 2 if the environmental temperature is placed in the model ($p < 0.05$). This means no difference was found between the temperatures measured on day 1 and day 2. Altogether, these findings suggest that IRT is not able to diagnose subclinical mastitis and can not be used as a quarter level diagnostics method to determine dry off treatment.

Introduction

Mastitis is one of the most common problems in the dairy industry and is responsible for large economic losses. Mastitis is mainly caused by bacterial intramammary infections (IMI). Inflammation of the mammary gland results in pathological changes in milk-secreting epithelial cells which results in decreased functional capacity. In cases of subclinical mastitis, the inflammation does not cause visible changes in the udder appearance or milk. In cases of clinical mastitis however, the affected quarter and/or the milk produced by that quarter are clinically abnormal. Changes in the milk can be change in colour, a watery or bloody appearance and the presence of pus, flakes and clots. An affected quarter can show symptoms such as swelling of the udder, heat, redness, edema and or/pain (Sharma et al., 2010). Mastitis is generally caused by infectious agents, mainly being bacteria. Inflammation of the mammary glands can be caused by infectious agents, chemical, physical or traumatic factors. Infectious microorganisms causing mastitis can be categorized as contagious and environmental pathogens. Pathogens characterized as contagious generally spread from animal to animal, from quarter to quarter, mainly during milking, through the milker's hands, milking machine components or miking cloths. Important contagious pathogens are *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma* and *Corynebacterium* (Kerro Dego et al., 2002). Environmental pathogens invade the udder directly from the environment and are found in bedding, soil or any pasture with which the cow comes in contact. Known environmental bacteria are *Escherichia coli* and *Streptococcus uberis* (Kalińska et al., 2017).

Most IMI are caused by bacteria that overcome the anatomical-physical barrier of the teat canal. Contact between bacteria entering the mammary gland and the somatic cells in the milk and lining epithelial cells results in inducting of the innate immune system (Wellnitz and Bruckmaier, 2012). The physical barrier of the mammary gland is formed by the teat canal and the surrounding musco-elastic tissue. The epithelial cells lining the teat canal continuously produce keratin. Keratin physically plugs the teat canal, which results in trapping the invading microbes. Keratin also produces bactericidal fatty acids and proteins (Rainard and Riollet, 2006; Smith, 2014). During milking, the vacuum and milk flux cause the keratin plug to flush out and the teat canal to distend. In between milkings, the sphincter muscles maintain a closed teat canal.
Recruited and resident cells in the mammary gland play an essential role in the immediate defense against local infection (Carrillo-Casas and Miranda-Morales, 2012). The primary immunity cells responding to invasion of pathogens are polymorphonuclear cells (PMN's). These phagocytes rely on an array of soluble mediators to recognize the presence of a pathogen. They include complement, acute phase proteins and lactoferrin (Smith, 2014). Lactoferrin binds iron and prevents multiplication of iron-dependent microorganisms, such as coliform bacteria. Acute phase proteins, such as C-reactive protein and mannose-binding lectin, will bind to the pathogen cell wall and hereby activate the complement cascade. An important role in gene expression and the release of inflammatory cytokines from the host cell are toll-like receptors. Toll-like receptors are resident on cell surfaces and within host cells (De Schepper et al., 2008). The actual number of toll-like receptors is important for the initiation of the immune response. In response to the inflammatory cytokines, neutrophils present in the blood will migrate into the milk. During IMI neutrophils often exceed concentrations of 1 million cells per milliliter of milk. Neutrophils have cytoplasmic granules which contain defensins with a broad-spectrum antibacterial and antifungal activities (Smith, 2014).

During the dry period, especially at the start and the end of that period, mammary glands of dairy cows are susceptible to the invasion of udder pathogens. Acute clinical mastitis rarely occurs in dry cows, but IMI gained during the dry period is a risk factor for the development of clinical mastitis after calving (Bradley and Green, 2004). Intramammary infections existing during the dry period can be divided into two groups. Existing IMI, which were already present at drying off and new IMI which occur during the dry period.

Risk factors related to the invasion of pathogens during the dry period include the level of milk production at the moment of drying off, the teat end condition, involution of udder and level of contamination of the udder and teat end (Nakov et al., 2014). The presence of teat-end lesions also plays an important role in the entry of pathogens into the mammary gland (Bhutto et al., 2010). Trauma of the teat-ends often occurs during milking because of poorly fitting cup liners, low vacuum reserve, excessive temporary vacuum losses, abrupt removal of the milking unit without shutting off the vacuum and excessive milking vacuum. An increased amount of teat-end callosity leads to a surface to which bacteria can easily adhere. Disinfecting the teat-end after milking is more difficult in this case. A study from (Bhutto et al., 2010) showed no clear association between IMI and the udder shape, but compared to other udder shapes, *S. aureus* and *S. uberis* were significantly less common in cows with larger hind quarters, possibly due to higher milk production.

Staphylococcus aureus is considered a major pathogen related to mastitis with a widespread prevalence. *Staphylococcus aureus* is a natural inhabitant of the skin of cows. It colonizes onto the skin of the mammary gland and reaches into the gland through the canal. It may cause subclinical and clinical mastitis (Kerro Dego et al., 2002). Peracute mastitis as a result of *S. aureus* IMI rarely occurs but is possible during the early lactation when the immune system of the cow is depressed. Clinical symptoms related to this form of mastitis are depression, a high fever, inappetence, death within 24 hours of the onset of symptoms. The infected quarter is swollen, very painful with blood-stained, serous secretion from the infected quarter (Webster, 2020).
A more common form of *S. aureus* mastitis is subclinical mastitis, which is less severe but chronic with sometimes clinical flare-ups. This type of *S. aureus* mastitis is associated with the production of a biofilm (Moormeier and Bayles, 2017). Due to the intracellular localization of the organism in mammary gland epithelial cells, response to antibiotic treatment is often low (Raza et al., 2013).

Streptococcus agalactiae is an obligate pathogen of the bovine udder. Infection results in most cases in subclinical mastitis. In the case clinical symptoms are present they are mild, with a slightly elevated temperature and limited loss of appetite (Webster, 2020). Persistent IMI destroys the alveolar cells which are replaced by scar tissue. This results in a decreased ability to produce milk. The SCC and bacteria counts in the milk are usually higher in IMI with *S. agalactiae* in comparison to *S. aureus* (Keefe, 2012). Due to specific control programs the prevalence of *S. agalactiae* in the Netherlands is low (Sampimon et al., 2009). *Streptococcus. agalactiae* generally is sensitive to antimicrobial therapy.

Another group of contagious mastitis pathogens is *Mycoplasma spp.,* of which *Mycoplasma bovis* is most prevalent. It usually presents as a chronic mastitis where the infected cow presents recurrent clinical episodes. *Mycoplasma spp.* produce no cell wall and possess less genetic code than other bacteria. This results in difficulty to identify the pathogen (Fox et al., 2005) and to treat it (Nicholas and Ayling, 2003). Several species of *Mycoplasma* are cofactors or actual causes of diseases of the respiratory tract, joints and mammary gland of the bovine. The presence of Mycoplasma pneumonia or arthritis in a herd is associated with the occurrence of *M. bovis* IMI (Nicholas et al., 2016).

The most important environmental pathogen related to mastitis is *E. coli*. Clinical signs of *E. coli* mastitis can vary between mild inflammation in one quarter with no systemic signs, to severe clinical signs and a decrease in the milk production (Burvenich et al., 2007). The severity of *E. coli* mastitis is dependent on the growth of *E. coli* bacteria during the acute phase before the influx of neutrophils. Intramammary infections are often of short duration and spontaneous elimination of *E. coli* is generally high. In cases of severe peracute *E. coli* mastitis, there is a potential risk of uncontrolled increase in bacteria growth in the milk. This may result in development of bacteremia. Therefore, it may be necessary to apply antimicrobials to reduce the number of bacteria (Suojala et al., 2013).

Sources of *S. uberis* in the environment of the bovine udder include bedding material, body sites and pastures. New IMI of *S. uberis* can occur during any stage of lactation, including dry cows and heifers before calving (Zadoks et al., 2003). *Streptococcus uberis* also is a major cause of subclinical mastitis, while only a small proportion of clinical cases are caused by *S. uberis.* One third of the IMI associated with *S. uberis* persists for a long period, two thirds of the IMI are cured within 30 days.

Antimicrobial use

Antimicrobial resistance (AMR) is a growing threat to human and animal health. Inappropriate use of antimicrobials in animal healthcare is considered to be responsible for the resistance increase in bacteria (Hyde et al., 2017). Susceptible bacteria are killed or inhibited by antimicrobials. Bacteria that are naturally resistant or have acquired AMR may survive treatment with antimicrobials and result in spreading the disease. Limiting the AMR can be reached by limiting the inappropriate use of antimicrobials. In the year 2008, the approach to the use of antimicrobials in veterinary medicine changed drastically (Lam et al., 2020). In the period 2009 to 2015, the antimicrobial usage (AMU) in the Dutch dairy sector decreased with 47%. Since 2015 the usage of antimicrobials has been stable. Mastitis is a major cause of antimicrobial use in cattle of which dry cow treatments are a substantial part. The decision if antimicrobials are used at drying off is currently generally made using diagnostics on cow level. In recent years AMU significantly decreased through the approach of selective dry cow treatment (Scherpenzeel et al., 2016). A next step would be to make treatment decisions on quarter level, this may result in lower AMU and AMR.

The dry-off antibiotic treatment has a dual target: cure the existing IMI and prevention of new IMI during the dry period. The dry cow therapy has consequences for the udder health during the dry period and during lactation. However the preventive use of antimicrobials is questionable, considering the risk of antimicrobial resistance of bacteria and is no longer allowed in the Netherlands (Santman-Berends et al., 2016).. In the period 2005 to 2010, approximately 90% of all dairy cows were treated with dry-cow antimicrobials (Lam et al., 2013). Antimicrobial use for the dry cow therapy was 49% of the total antimicrobial use in the Dutch dairy industry. Preventive use of antimicrobials is no longer allowed in several European countries (Scherpenzeel et al., 2016). At this time antimicrobial use at the moment of drying off is only allowed for the curative use after IMI is diagnosed. The SCC on cow level, determined a maximum of six weeks before drying off, is in most cases the standard diagnostics for diagnosing IMI. In the case of a heifer the SCC should be above 150.000 cells/ml to use antimicrobials at drying off. In case of a multiparous cow, the SCC should be above 50.000 cells/ml (KNMvD, 2013).

Intramammary injectors used at drying off contain first choice antibiotics, which are allowed to be included in the treatment plan. Second choice antibiotics drying injectors are allowed to be used after a written plan of approach using the history over the last three months (KNMvD, 2013). In the case of third choice antibiotics, a 'no, unless' approach is used. These antibiotics are only allowed to be used after bacterial research and if there are no other options available. In addition to antimicrobials, teat sealants are used to reduce the risk of new IMI during the dry period (Webster, 2020). A teat sealant generates a latex, acrylic or other polymer-based film in the teat end which prevents the entry of new pathogens during the dry period (Godden et al., 2003).

Diagnostics of IMI

Identification of IMI at drying off can be based on somatic cell count (SCC) and bacterial culture. Diagnostics for identification of IMI can be divided into diagnostics on cow-level and diagnostics on quarter-level. The most common method for monitoring the prevalence of mastitis is the SCC. It measures the number of cells per milliliter of milk, primarily macrophages and leukocytes, which reflects the immune response. The SCC is important for detection of IMI and subclinical inflammation. In uninfected glands, the SCC is below 200.000 cells/mL. A change in the SCC from <200.000 to > 200.000 is a good indicator for the presence of inflammation (Dohoo and Leslie, 1991). Treatment at the moment of drying off can be determined by the SCC on cow level measured at the milk production registration (MPR). Increase in the SCC is in most cases explained by IMI, but heat stress, stage of lactation and the hormonal cycle of the cow are also influencing SCC (Windig et al., 2010). This means that stage of lactation, parity and the season should be taken into account when interpreting the SCC. Subclinical mastitis in one quarter may be missed with a SCC measurement on cow level, because the higher SCC may be diluted by the lower SCC of the healthy quarters.

A SCC above 200.000 on quarter level is not always combined with a positive bacterial culture. The SCC is related to the immune response (the influx of leukocytes), the immune response is often effective in reducing the presence of bacterial colonies in the milk (Smith, 2014).

Systematic treatment of all mammary quarters at time of drying off has been effective for the treatment and prevention of IMI. Using diagnostics on quarter level, performed on the farm before drying off, to determine the treatment plan on quarter level could reduce the antimicrobial usage. An example of a diagnostic method on quarter level is the California Mastitis Test (CMT). CMT is easy to perform and low in cost. The CMT reagent is a mixture of a detergent and bromocresol purple, which is used as an indicator of the pH (Smith, 2014). The degree of reaction between the detergent and DNA of cell nuclei is a measurement of the number of somatic cells in the milk. It is important to note that the concentrations of white blood cells and DNA are directly correlated with each other and can be used to estimate the number of WBCs in the sample. The purple color is generally more intense in milk samples from infected quarters, the deep purple color indicates abnormal composition of the milk. Depending on the leukocyte count per milliliter, the test is scored from negative to 3+. The CMT qualitatively measures the amount of DNA in the milk secretions. This test is most useful in detecting subclinical mastitis and (Divers and Peek, 2008). Correct use and rating are dependent on the person executing the CMT. No exact value of the SCC is predicted with CMT. Other diagnostics methods on quarter level are lactate dehydrogenase measurements in a milk sample, bacterial culture, SCC on quarter level and measuring the electrical conductivity of the milk.

Infrared thermography

Infrared thermography (IRT) is a simple, effective, and non-invasive method that detects surface heat. Surface heat is generated as a pictorial image without radiation exposure. A thermogram shows warm regions as white or red and cool regions as blue or black (Sathiyabarathi et al., 2016). In veterinary medicine, IRT has been described for identifying areas of inflammation for example in detection of lameness in cows and in the detection of heat and ovulation (Alsaaod and Büscher, 2012; Talukder et al., 2014).
IRT has been shown to be able to detect clinical mastitis in an experimental setting. A significant increase in the average and the maximum temperature in the affected quarter was measured (Metzner et al., 2015; Hovinen et al., 2008). In subclinical mastitis a difference of 2.35 °C in temperature between healthy and infected quarters has been found (Polat et al., 2010). Local signs of IMI, results in an increasing permeability of the capillaries and plasma will leak into the interstitium. Causing edema, this will result in an impaired blood circulation and decrease of the local temperature. This might explain why local signs of IMI could not be detectable by IRT if severe swelling is present (McGavin and Zachary, 2007).

In two studies it was shown that detection of mastitis using IRT was only successful in the hind quarters (Metzner et al., 2014; Berry et al., 2003). The temperature of the front quarters is affected more by thermal radiation from the medial side of the adjacent legs in comparison with the hind quarter surface temperature. In a study by Polat et al., (2010), as expected, no difference in the surface temperature was found among four non-infected quarters.

The influence of wind on the IRT measurements is investigated by Church et al. (2013). IRT temperature readings were red from the left eye of cattle. The IRT temperature reading decreased from the baseline at wind speeds of 7 km/h and decreased even further at wind speeds of 12 km/h. Udder skin temperatures are under the influence of the environmental temperature. The study from Church et al., (2013) found an increase in the IRT temperature of the eye in direct sunlight by 0.56 ± 0.36 °C.

Aim of the study

IRT is a noninvasive and rapid method, which seems easily applicable on the farm and can be an important tool in addition to techniques available for mastitis diagnostics. In the current approach of optimizing antimicrobial use in dry cow treatments in dairy cows a next step may be to make dry cow treatment decisions at quarter level rather than on cow level. In order to make that practically applicable, an easy diagnostic method such as IRT may be of added value. The aim of the study therefore is to determine if IRT can be used to diagnose subclinical mastitis on quarter level. A higher surface temperature is expected in the case of an infected quarter and a significant association between the temperature and SCC and a significant association between the temperature and positive bacterial growth are expected.

Materials and Methods

<u>Materials</u>

A total of 53 dairy cows at the start of the dry period from three different dairy farms were selected. The farms are selected based on the number of dairy cows (> 200 dairy cows). Unfortunately, cows could not be selected based on their SCC on cow level due to the small number of cows available and the limited time. Dairy cows with clinical mastitis at the start of the dry period were not included into the study.

The IRT pictures were taken within 24 hours, but in most cases not on the same day as the start of the dry period. No analysis of whether a difference in quarter temperature was present between the day of drying off (day 0) and the day after (day 1), a total of 13 dairy cows were photographed at day 0 and day 1. Dairy cows from two dairy farms were selected. The SCC on cow level was given by the farmer but no additional information was available about the 13 dairy cows used for this part of the study.

<u>Methods</u>

The SCC is measured at the last MPR (milk production registration) before the start of the dry off period. Milk samples for the bacterial culture and SCC on quarter level were collected by the farmer at the moment of drying off (day 0). Additional information about the dairy cows, such as milk production in the last 24 hours, is given by the farmer. Environmental temperature and humidity are noted on the day the IRT pictures are taken. In addition the udder pressure of the hind quarters is measured using an algometer, as the udder pressure may be a variable which influences the udder temperature.

IRT pictures were taken within 24 hours after drying off, but often not on the same day as the start of the dry period. IRT pictures were taken at a distance of 0.5 meter and 1 meter of dairy cows in a standing position. Images of the two front quarters are made from the lateral side and images of the two hind quarters can be made from the lateral or posterior side (Colak et al., 2008; Polat et al., 2010). The images were not taken in direct sunlight.

IRT images were taken with a Testo 881 thermal imager. Professional IRSoft thermography analysis software is used for analysis of the images and to determine the maximum temperature, the location of the maximum temperature and the mean temperature of each quarter. The average temperature is measured by taking two pictures per quarter, one at a distance of 0.5 meter and the other one at a distance of 1 meter. Based on the possible difference between the udder temperatures at a distance of 0.5 meter and a distance of 1 meter, the best applicable distance of taking the IRT picture is analyzed. The IRT picture is analyzed using a polygon measuring tool, which means the entire quarter including the teat end is analyzed.

The location of the maximum temperature is analyzed using the polygon measurement tool. Using the polygon measurments tool, the quarter is manually outlined. Within the lines the temperature is measured. The quarters are divided into sections. In the case of the hindquarter the quarter was divided into quarter in contact with hindleg (section 1), above teat end (section 2), teat end (section 3), in contact with the median intermammary groove (section 4), in contact with the rear udder attachments (section 5) or the middle of the quarter (section 6). The front quarter was divided into quarters in contact with hindleg (section 1), above teat end (section 2), teat end (section 3), in contact with abdomen (section 4) or middle of the quarter (section 5). The frequency in which section the maximum temperature is found is noted.

To determine if a difference in temperature was present between the day of drying off and the day after, in a total of 13 cows IRT pictures were taken on the day of drying off and the day after. The IRT picutres were analyzed using the polygon measurement tool. To ensure no large increases or decreases in udder quarter temperatures are found between day 1 and day 2, two more ways of analyzing the IRT pictures are examined. The first method is to only analyze the region above the teat end and the second method is to analyze the entire quarter but place a line further from the outside of the quarter to ensure heat radiating from the hind leg is not interfering with the temperature measured.

Because the difference in temperature between 0.5 meter and 1 meter is found to be minimal and a practical use is preferred, the main part of the analysis will be done with the temperatures measured at 1 meter distance. At 1 meter distance the entire quarter will be visible in the IRT pictures, this means the entire quarter temperature will be measured.

<u>Analysis</u>
Data analysis is performed using Stata 15.1. Outcomes of interest were the temperature of the different quarters, bacterial culture, SCC on quarter level and SCC on cow level. Variables of possible influence of the udder temperature were noted, which were the milk production, presence of draft, the duration of the period standing before the IRT picture was taken, humidity, environmental temperature and udder pressure. The duration of the standing period was turned into a 0/1 value. If the dairy cows stood for a period longer than 30 minutes, the duration of the standing period before the IRT picture was taken was considered long (1). If the period was shorter than 30 minutes, the period was considered short (0). The cut-off value of 30 minutes was based on the amount of time between arriving at the dairy farm and taking the IRT picture. These cow- and environmental variables may be of influence on the mean and maximum temperature measured. To determine if there is a significant association, first univariate analyses were performed. To correct for correlation between observations in udder temperatures in udder quarters from the same cow, multilevel regression analyses (multilevel mixed-effects linear regression) were conducted. Data was analyzed on quarter level taking cows into account as a random effect. If any of the P-values are below 0.3, these variables will be analyzed in a multivariate multilevel regression model. These analyses are performed only on the dairy cows without any IMI, to ensure any possible influence of the present intramammary infection is not included.

First a univariate multilevel regression model is performed with the mean and maximum udder temperatures and the SCC on cow and quarter level to detect a possible significant association between the udder temperatures and the SCC. The same univariate multilevel regression model is performed with the mean- and maximum temperature and the different variables of possible influence. If the p-value is below a 0.3, variables are taken into a multivariate multilevel regression model to determine a significant association. Outcomes with a p-value below 0.5 were considered significant.

The bacterial culture outcome is translated into a 0/1 value called IMI and subclinical mastitis. For the first variable a quarter was considered positive for intramammary infection when the bacterial analysis was positive. When there was no bacterial growth, the quarter was considered negative for intramammary infection. The second variable is called subclinical mastitis and this variable also focuses on the bacteria which were found during the bacterial analysis. The quarters which are infected with bacteria which could benefit from antibiotic treatment during the dry period are considered positive for subclinical mastitis. Bacteria found which could benefit from antibiotic treatment were *S. aureus, S. uberis, S. chromogenes, S. haemolyticus, viridans streptococci, S. sciuri, S. equorum, Lactococcus garvieae* and *S. dysgalactiae spp. dysgalactiae*. Quarters infected with *Corynebacterium bovis* or *Enterococcus faecium* are not considered positive for subclinical mastitis as were quarters with no bacterial growth.

First a univariate multilevel logistic model is performed with the iIMI variable and the mean- and maximum temperature. The same is repeated with the SCC on cow- and quarter level and the presence of IMI. Next a univariate multilevel logistic model is performed with the different variables of possible influence. If the p-value of any of the variables is below 0.3, the variables are taken into a multivariate multilevel logistic model to determine if a significant association is found between the presence of an intramammary infection (variable of interest) and any of the tested variables. The same steps are followed using the subclinical mastitis variable.

To determine if a difference in udder temperatures between the day of drying off (day 1) and the day after (day 2) is present, an univariate multilevel regression model is performed using the quarter temperature measured on both days. A p-value below 0.5 showed no difference in udder temperature between day 1 and day 2. A multivariate multilevel regression model was performed with the quarter temperatures measured at day 1 and day 2 and the difference in environmental temperature between both days.

Results

Descriptive statistics

The mean and maximum udder quarter temperatures of a total of 53 dairy cows at the moment of drying off were measured. Between 9 and 25 cows per dairy farm were evaluated, a total of 3 dairy farms were visited. The included dairy cows all had four productive quarters.

Mean quarter temperature

The mean quarter temperature was measured at a distance of 0.5 meter and 1 meter. The mean quarter temperature at 0.5 meter distance varied between 26 °C and 36.8 °C (mean: 31.7 °C, median: 31.8 °C). The quarter temperature measured at a distance of 1 meter varied between 26.5 °C and 40.3 °C (mean: 31.6 °C, median: 31.8 °C). The mean quarter temperature udder quartile was calculated using the mean temperatures measured at 0.5 meter and 1 meter. The quarter temperature varied between 26.3 °C and 36.8 °C (mean: 31.6, median: 31.85). The mean quarter temperatures measured at a distance of 0.5 meter and 1 meter were normally distributed.

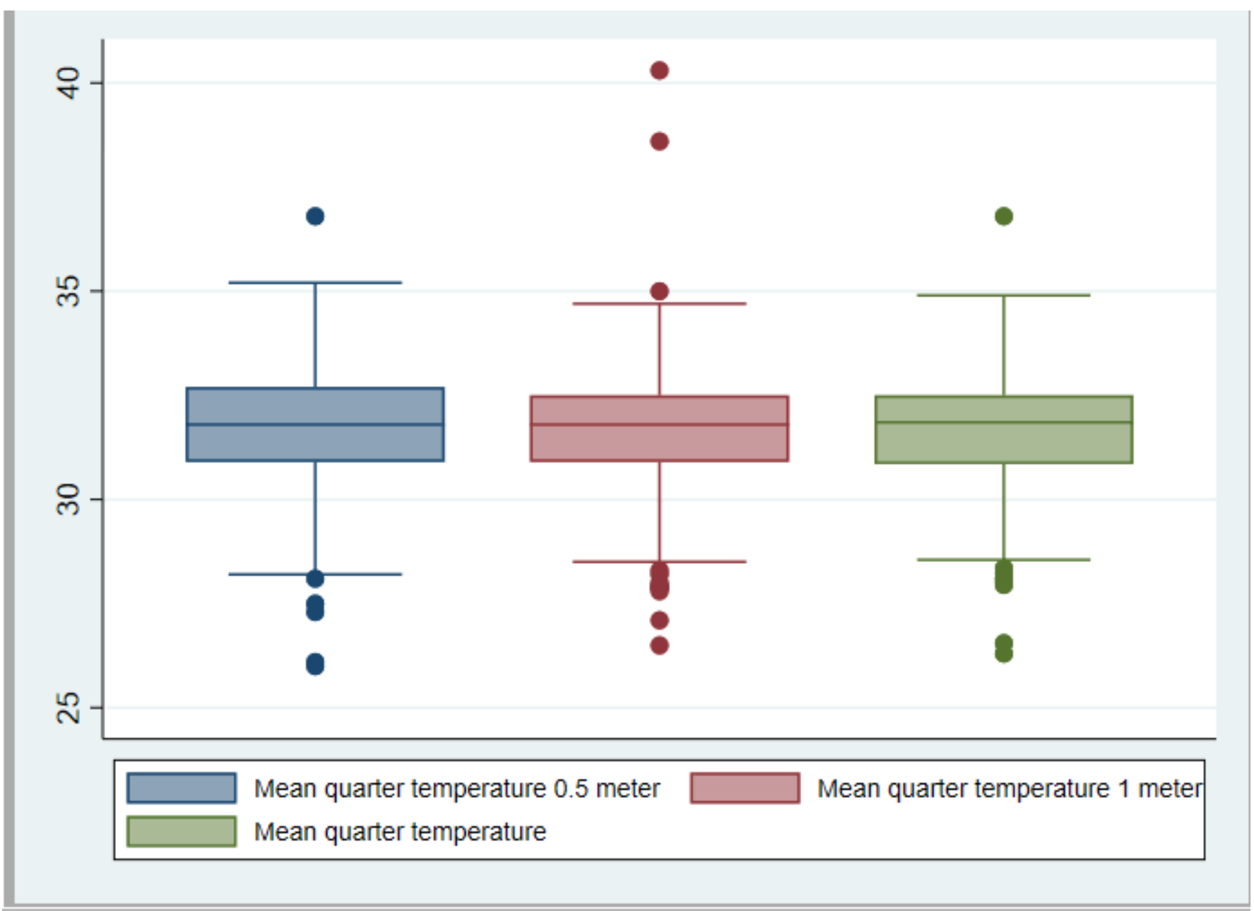

Figure 1: Boxplot representing the variance in mean quarter temperature (n=212). The mean quarter temperature at a distance of 0.5 meters varied between 26℃ and 36.8℃. The mean quarter temperature at a distance of 1 meter varied between 26.5℃ and 40.3℃. The mean temperature of the quarters varied between 26.3℃ and 36.8℃ with a mean of 31.6℃ and median of 31.9℃.

Maximum quarter temperature

The maximum temperature of the quarters was measured at a distance of 0.5 meter and 1 meter. The maximum quarter temperature measured at 0.5 meter distance varied between 30.6 °C and 39 °C (mean: 35.0 °C, median 35.0 °C). The maximum quarter temperature measured at 1 meter distance varied between 29.2 °C and 43.3 °C (mean: 34.9 °C, median: 34.9 °C). The maximum temperature per quarter was calculated using the average of the maximum temperatures at 0.5 meter and 1 meter. The maximum quarter temperature varied between 29.9 °C and 40.0 °C (mean: 34.9 °C, median: 34.95 °C). The maximum temperatures measured at a distance of 0.5 meter and 1 meter were normally distributed.

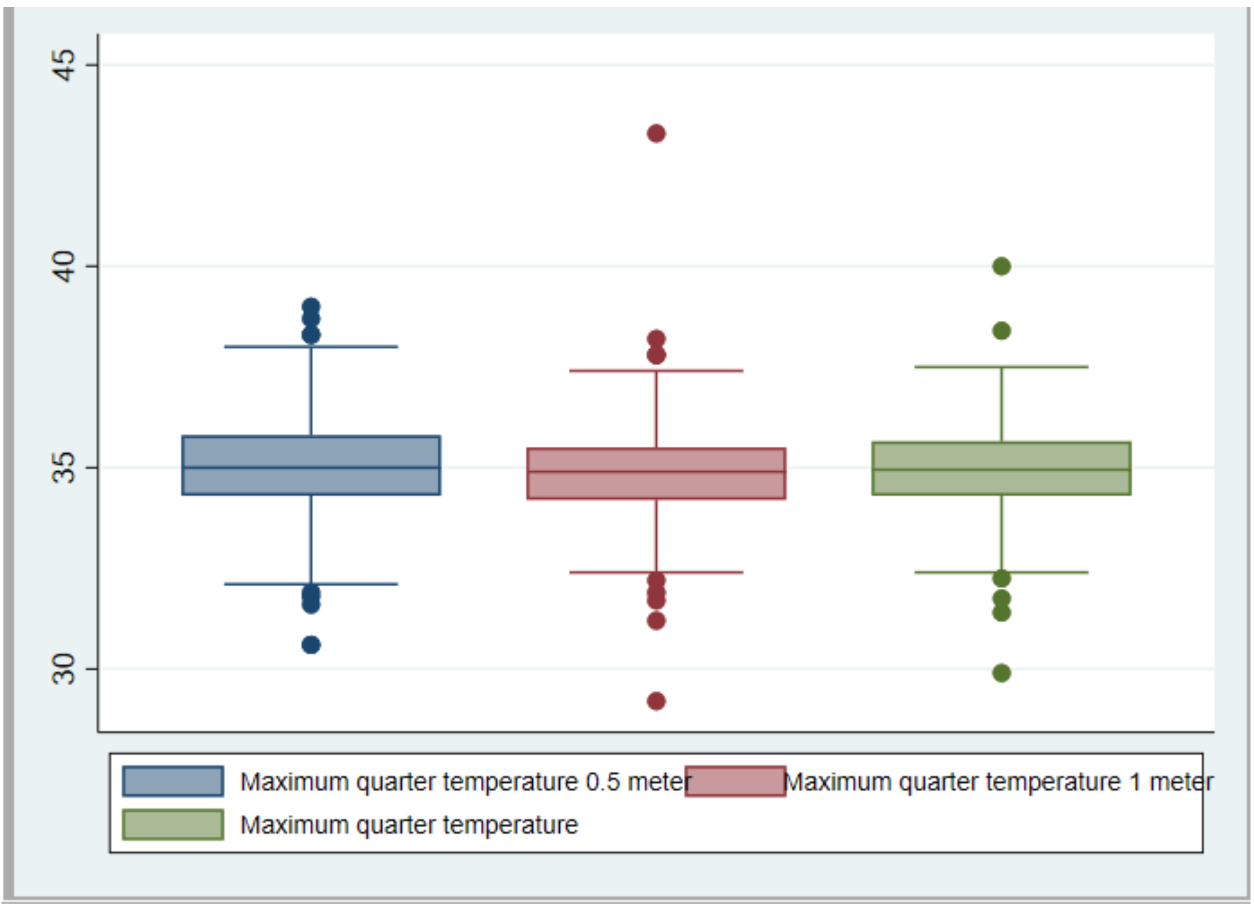

Figure 2: Boxplot representing the variance in quarter temperature measured at 0.5 meter and 1 meter. The maximum quarter temperature measured at 0.5 meter distance varied between 30.6℃ and 39.0℃. The maximum quarter temperature measured at 1 meter distance varied between 29.2℃ and 43.3℃. The maximum temperature per udder quarter varied between 29.9℃ and 40.0℃.

To determine the most practical way to use the IRT camera, the correct distance to take the IRT picture should be analyzed. The mean difference between the mean temperature measured at 0.5 meter versus the temperature measured at 1 meter of the same quarter is 0.03 °C (max: 9.7 °C, med: 0 °C) (figure 3). The mean difference between the maximum temperature measured at 0.5 meter and the maximum temperature measured at 1 meter of the same quarter is 0.09 °C (max: 6.6 °C, med: 0.1 °C). Because the difference in temperature between 0.5 meter and 1 meter is minimal and a practical use is preferred, the rest of the analysis will be done with the quarter temperatures measured at 1 meter distance. At 1 meter distance the entire quarter will be visible in the IRT pictures, this means the entire quarter temperature will be measured.

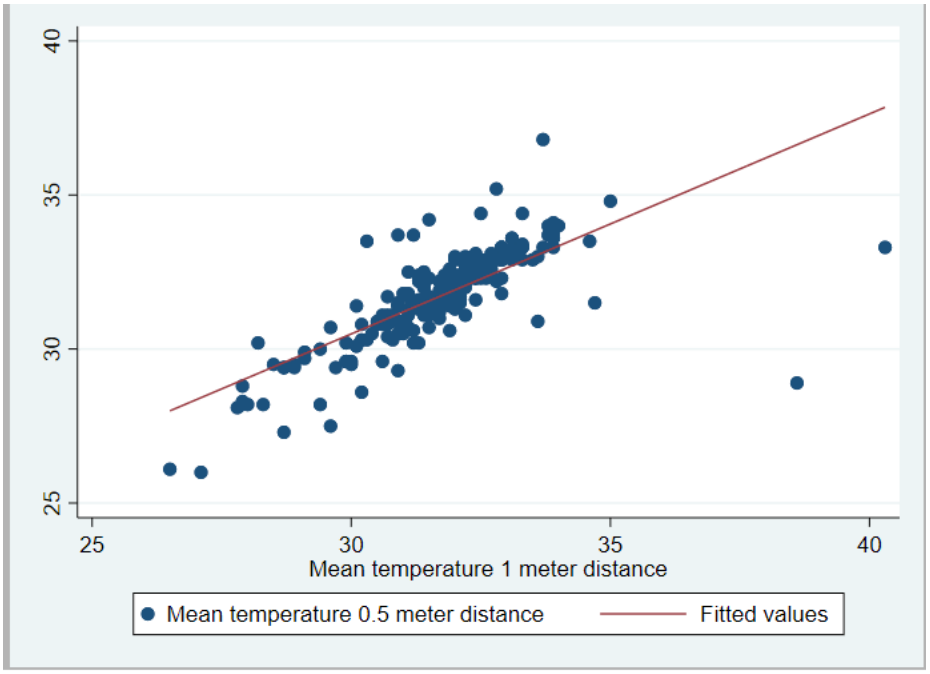

Figure 3: Scatterplot representing the mean quarter temperature measured at 0.5 meter and 1 meter. The linear distribution of the graph shows that if the quarter temperature measured at 0.5 meter is higher, the quarter temperature measured at 1 meter is also higher. The red line shows the linear relationship between the two quarter temperatures measured.

<u>Location of the maximum temperature</u>

The location of the quarter where the temperature is at its highest is examined. The quarters are divided into sections. In the case of the hindquarter the quarter was divided into quarter in contact with hindleg (section 1), above teat end (section 2), teat end (section 3), in contact with the median intermammary groove (section 4), in contact with the rear udder attachments (section 5) or the middle of the quarter (section 6). The front quarter was divided into quarters in contact with hindleg (section 1), above teat end (section 2), teat end (section 3), in contact with abdomen (section 4) or middle of the quarter (section 5).

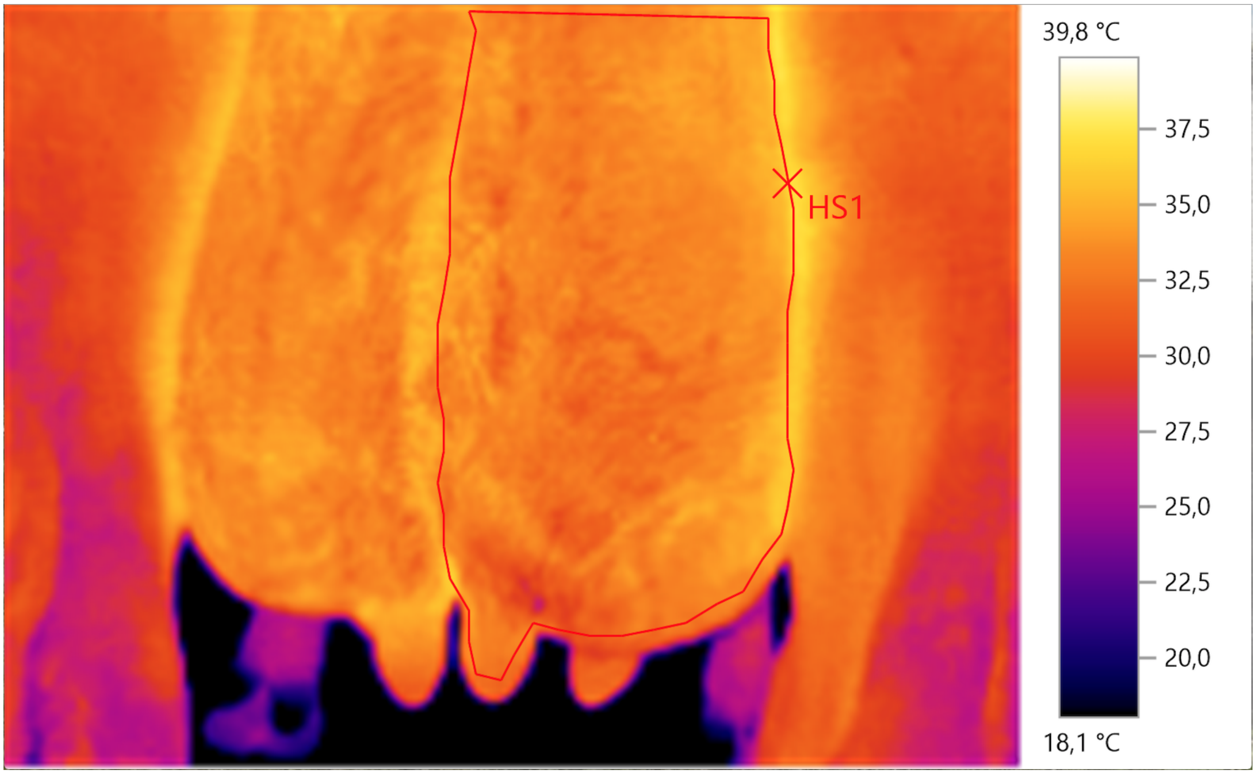

Image 1: IRT image of the hind quarters taken from 1 meter distance. The HS1 shows the maximum temperature location. In this case the maximum temperature measured on the quarter is in contact with the hind leg. The red line shows the region in which the temperature is measured.

Location maximum temperature	Frequence (hindquarters)	Percent (hindquarters)	Frequence (front quarters)	Percent (front quarters)
1	*59*	*55.66*	*85*	*80.19*
2	*0*	*0*	*2*	*1.89*
3	*0*	*0*	*0*	*0*
4	*43*	*40.57*	*6*	*5.66*
5	*1*	*0.94*	*13*	*12.26*
6	*3*	*2.83*	-	-

Table 1: Frequency and percentiles of the location of the maximum temperatures measured.

As seen in table 1, in the front quarters, the maximum quarter temperature was most often found where the quarter was in contact with the hind leg. The teat end was in almost all cases the coldest location on the front quarter. In the case of the hindquarters, the maximum temperature was most often measured where the quarter came in contact with the hind leg or next to the intramammary groove.

Environmental variables and cow level variables

The quarters were rated on the amount of dirt covering the udder skin. Mud, scobs or feces covering the udder skin show up colder on the IRT picture which influences the mean temperature measured. Removing the dirt beforehand could influence the temperature of the udder skin. A total of 172 quarters were considered clean and 40 quarters were considered unclean due to the presence of feces, mud of scobs. The duration of standing before the IRT picture was taken was noted. If the cow stood for a period longer than 30 minutes, the duration was considered 'long'. If the duration was shorter than 30 minutes, the standing period was considered 'short'. The duration of standing was taken into account because of the expectation that if the cow lay down on one side for a longer period of time, this may result in a higher quarter temperature of the quarters in contact with the floor. A total of 68 cows stood for a short period and 144 cows stood for a longer period. Draft could possibly be of influence on the temperature measured with the IRT picture. Draft was present in 32 cases and in 180 cases no draft was present.

The milk production (mean 17.9, median 17.3) in the last 24 hours before drying off was listed. The highest milk production measured was 33 liters in 24 hours, the least milk production 5.6 liters in 24 hours. The pressure of the quarters was measured in the hind quarters. The lowest udder pressure measured was 5.9 N, the highest udder pressure measured was 30.4 N (mean: 16.5 N, median: 15.05 N).

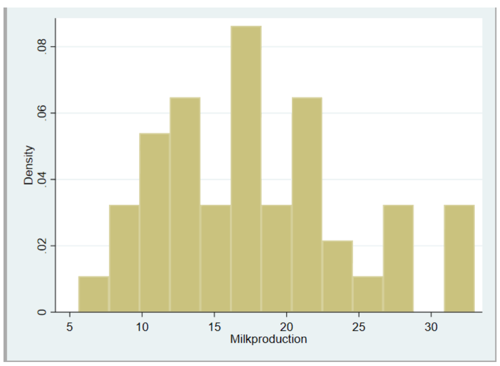

Figure 4: The milk production is the last 24 hours before drying off.

The final two variables of possible influence are the environmental temperature and humidity when the IRT picture is taken. The outside environmental temperature and humidity are noted. The environmental temperature varied between 4 ℃ and 20 ℃ (mean 13.3 ℃, median 15 ℃). The humidity varied between 49% and 98% (mean: 84.1%, median: 86%). Using a median test (humidity does not have a normal distribution), an association between humidity and draft is found (p = 0.000).

The univariate analyses in which the association with the mean udder temperature was analyzed, resulted in P-values below 0.3 for the following variables: the environmental temperature (p = 0.000), milk production (p = 0.008), udder pressure (p = 0.060) and the amount of dirt covering the udder skin (p = 0.130). From the multivariate analysis only a significant association between the mean quarter temperature and the environmental temperature (p = 0.000) was found. The rise of the environmental temperature with 1 ℃ results in a rise of the mean udder temperature with 0.29 ℃ (95% confidence interval 0.18 - 0.41). Using a reverse stepwise analysis and a stepwise forward regression, the milk production and udder pressure also give a significant multivariate model (p = 0.008) if the environmental temperature is not present in the multivariate model.

The significance of the variables on the maximum quarter temperature was also analyzed. The univariate analyses in which the association with the maximum temperature was analyzed, resulted in P-values below 0.3 for the following variables: the environmental temperature (p = 0.000), humidity (p = 0.160), milk production over the last 24 hours (p = 0.055), udder pressure (p = 0.058) and the amount of dirt covering the udder skin (p = 0.207). From the multivariate analysis only a significant association between the environmental temperature (p = 0.000) and the maximum quarter temperature was found. A rise in the environmental temperature with 1 °C results in a rise of the maximum udder temperature with 0.21 °C (95% confidence interval 0.1-0.33). Using a reverse stepwise analysis and a stepwise forward regression, the combination of the milk production and udder pressure also give a significant multivariate model (p = 0.036) if the environmental temperature is not present in the multivariate model.

<u>Relationship between the SCC and quarter temperature</u>

The SCC measured on quarter level varied between 11,000 cells/ml and 7,301,000 cells/ml (mean: 776,300 cells/ml, median: 298,000 cells/ml, n=208). In four milk samples the amount of milk was not enough to measure the SCC on quarter level. The SCC on quarter level was not significantly associated with the mean quarter temperature measured at 1 meter (p=0.346) (figure 5).

The SCC on cow level varied between 11,000 cells/ml and 2,920,000 cells/ml (mean 236,500 cells/ml, median: 83,000 cells/ml, n=180). There is also no significant association between the SCC on cow level and the mean quarter temperature (p = 0.776)

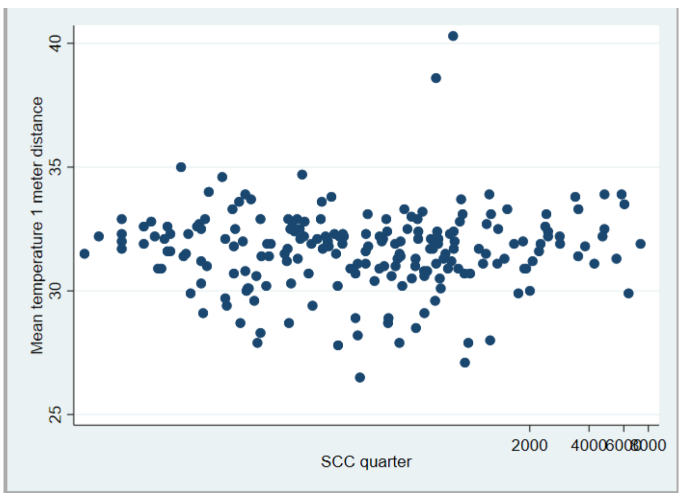

Figure 5: Scatterplot representing the correlation between the mean quarter temperature measured at a 1 meter distance and the SCC on quarter level. No significant association was found. The SCC on quarter level is plotted on a logarithmic scale.

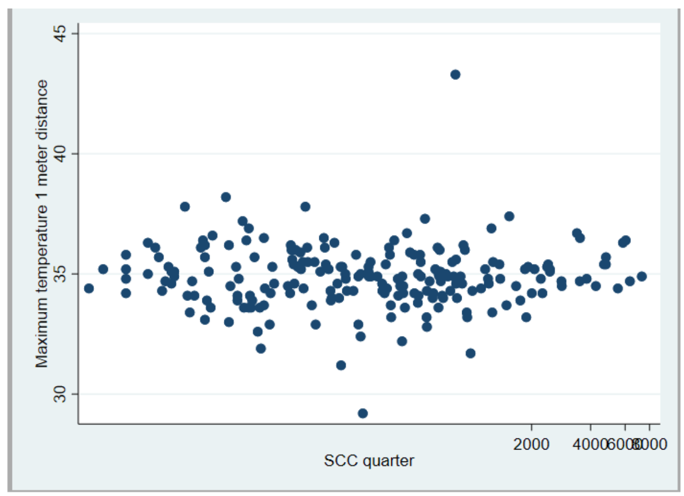

Figure 6: Scatterplot representing the correlation between the maximum quarter temperature measured at a 1 meter distance and the SCC on quarter level. No significant association was found. The SCC on quarter level is plotted on a logarithmic scale.

The significant association between the SCC on quarter level and the maximum temperature measured was also analyzed. At a distance of 1 meter, there is no significant association between the SCC on quarter level and the maximum quarter temperature (p = 0.263) (figure 6). The association between the maximum quarter temperature and the SCC on cow level registered at the last MPR is analyzed. There is also no significant association found between the SCC on cow level and the maximum quarter temperature measured at 1 meter distance (p = 0.635).

Subclinical mastitis

A sample for bacterial analysis was taken the day of drying off. The frequency of bacteria found during bacterial analysis is shown in table 2. A total of 171 bacterial growths are analyzed.

Bacteria found	Frequency
Negative bacterial culture	73
Mixed flora	37
Corynebacterium bovis	32
Staphylococcus aureus	27
Staphylococcus haemolyticus	16
Staphylococcus chromogenes	13
Enterococcus faecium	3
Viridans streptococci	3
Streptococcus dysgalactiae spp. dysgalactiae	2
Staphylococcus sciuri	1
Streptococcus uberis	1
Lactococcus garvieae	1
Staphylococcus equorum	1

Table 2: Table representing the frequency of bacteria found during bacteriological analysis of the milk samples taken at drying off. .

Because no clear bacterial outcome could be made from samples with the diagnosis 'mixed flora', these quarters were interpreted as having a 'missing value' (n=37). Bacterial cultures with two different bacteria, were placed into the group subclinical mastitis if one of the two was a major pathogen. No contaminated samples were found. Two types of variables were formed to analyze the association of the measured quarter temperature with the presence of subclinical mastitis.

For the first variable a quarter was considered positive for intramammary infection when the bacterial analysis was positive. When there was no bacterial growth, the quarter was considered negative for an intramammary infection. A total of 100 quarters has a positive outcome of the bacterial culture and 73 a negative outcome of the bacterial culture. The second variable is called subclinical mastitis and this variable also focuses on the bacteria which were found during the bacterial analysis. The quarters which are infected with a major bacteria which could benefit from antibiotic treatment during the dry period are considered positive for subclinical mastitis (n = 64). Bacteria seen in this group are *S. aureus, S. uberis, S. chromogenes, S. haemolyticus, viridans streptococci, S. sciuri, S. equorum, Lactococcus garvieae* and *S. dysgalactiae spp. dysgalactiae*. Quarters infected with *Corynebacterium bovis* or *Enterococcus faecium* are not considered positive for subclinical mastitis as were quarters with no bacterial growth (=109).

First the outcome of IMI is analyzed in which no distinction in bacteria is made. As expected, there is a significant association between the subclinical mastitis and the SCC on quarter level (p = 0.014). An increase with log 1 of the

SCC on quarter level leads to a 2.3 times higher chance of subclinical mastitis. There is no significant association between presence of the IMI and the SCC on cow level (p = 0.080), but a clear trend is visible.

Next the possible relationship between the measured quarter temperatures and the presence of IMI is examined. There is no significant association between the presence of IMI and the mean quarter temperature (p = 0.977). No significant association has been found between the presence of IMI and the maximum quarter temperature (p = 0.779).

The same analysis is performed with subclinical mastitis. A significant association has been found between subclinical mastitis and the SCC on quarter level (p = 0.001). There is not a significant association between presence of subclinical mastitis and the SCC on cow level (p = 0.053), but a clear trend is visible.
The mean quarter temperature (p = 0.822) and maximum quarter temperature (p = 0.597) are not significantly associated with the presence of subclinical mastitis.

Moment IRT picture is taken
The IRT pictures are taken within 24 hours after the actual drying off, being the next day. On day 1 the temperature at 1 meter distance varied between 29.1 °C and 34.7 °C (mean: 32.4 °C, median: 32.35 °C). The mean temperature at 1 meter distance on day 2 varied between 30.5 °C and 35.8 °C (mean: 32.7 °C, median 32.8 °C).

At a distance of 1 meter the maximum difference in quarter temperature between day 1 and day 2 is 4.2°C (mean: 0.3°C, median: 0.65°C). The null hypothesis would be that the difference between the mean quarter temperatures is equal to zero. The alternative hypothesis would be that the difference between the mean quarter temperatures is not equal to zero.
At a distance of 1 meter there is no significant association between the quarter temperatures at day 1 and day 2 (p = 0.080). A clear trend is visible. Because the environmental temperature has an influence on the udder temperature, a multivariate multilevel regression model is performed with the quarter temperature measured at day 1 and day 2 and the difference in environmental temperature between day 1 and day 2. In the multivariate model there is a significant association between the quarter temperature measured at day 1 and day 2 if the difference in environmental temperature is placed into the multivariate model (p = 0.002).

The maximum quarter temperatures are also measured on day 1 and 2 at the distance of 1 meter. At a distance of 1 meter the maximum quarter temperatures varied between 32.3 °C and 37 °C (mean: 35.0 °C, median: 34.85 °C). At a distance of 1 meter, the maximum quarter temperatures varied between 33.1 °C and 37.8 °C (mean: 35.1 °C, median: 35.1 °C). At a distance of 1 meter the maximum difference between the quarter temperatures at day 1 and day 2 is 3 °C (mean: 0.08 °C, median: 0.05 °C. No significant association has been found between the quarter temperatures at day 1 and day 2 (p = 0.429). A multivariate multilevel regression model is performed using the quarter temperatures measured at day 1 and day 2 and the difference in environmental temperature. A significant association (p = 0.001) is found between the quarter temperatures at day 1 and day 2 if the difference in environmental temperature is taken into account. This means there is no significant difference in the measured mean and maximum quarter temperatures between day 1 and day 2.

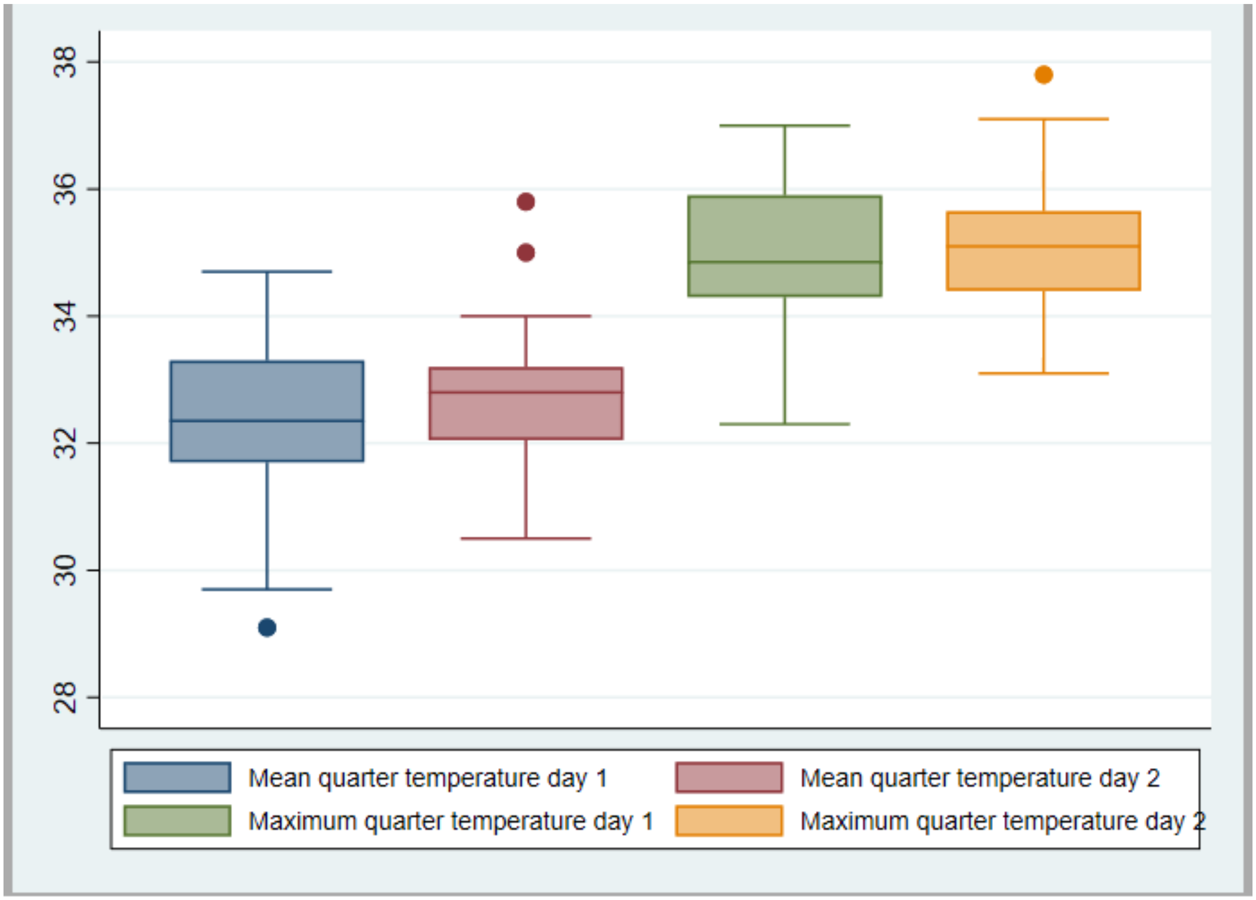

Figure 7: Boxplot representing the difference between day 1 and day 2 in the mean and maximum quarter temperature measured. On day 1 the dispersion between the temperatures measured is larger than on day 2. The temperature is plotted on the Y-axis.

To ensure no large increases or decreases are found in udder quarter temperatures are found between day 1 and day 2, two more methods of analyzing the IRT pictures are examined (image 2 and 3).
Firstly, the mean quarter temperatures above the teat end are analyzed using a univariate multilevel regression model. There is a significant association between the quarter temperatures above the teat end measured at day 1 and day 2 ($p = 0.000$). A rise in udder temperature with 1 ℃ on day 1 results in a rise in quarter temperature with 0.6 ℃ on day 2 (95% confidence interval 0.398 - 0.807). Adding the environmental temperature to the regression model, makes no difference in the p-value. The same univariate multilevel regression model is performed using the maximum quarter temperature measured. No difference in the maximum quarter temperature above the teat end between day 1 and day 2 is found ($p = 0.000$).

Secondly, the mean quarter temperatures of the entire quarter are analyzed using a multivariate multilevel regression model, using the difference in environmental temperature in the model. There is no significant difference in the mean quarter temperature between day 1 and day 2 ($p = 0.000$). A rise in the quarter temperature with 1 ℃ on day 1 results in a rise in udder temperature with 0.64 ℃ on day 2 (95% confidence interval 0.43-0.86). Also, no difference in the maximum quarter temperature was found between day 1 and ($p = 0.000$). A rise in the maximum quarter temperature on day 1 with 1 ℃ results in a rise of the maximum quarter temperature on day 2 with 0.5 ℃ (95% confidence interval 0.36-0.71).

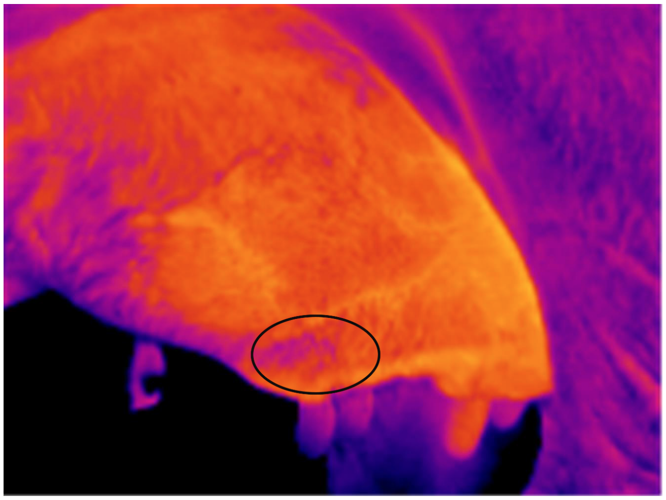

Image 2: IRT picture, this represents the first method in which the region above the teat which was analyzed.

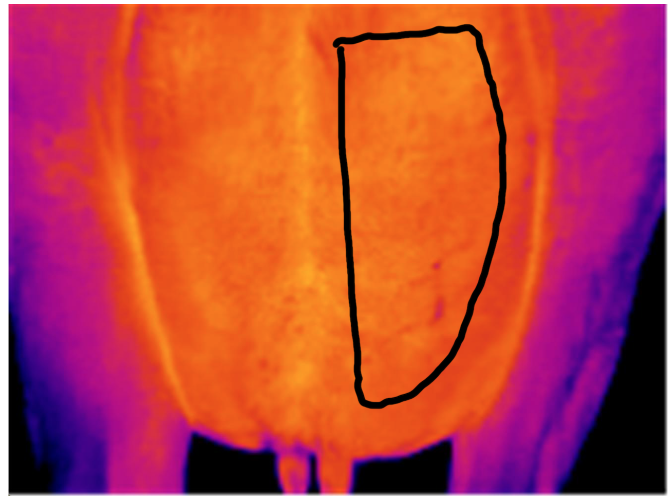

Image 3: IRT picture, the region in between the black lines shows the region analyzed in the second method.

Discussion

Early detection of subclinical mastitis is important for the composition of the treatment plan at drying off. The treatment plan for drying off is currently in most cases based on cow level diagnostics. In this study, IRT was tested to be a good quarter level diagnostics to diagnose subclinical mastitis at drying off.

In this study the quarter temperature was measured with IRT, as a tool to diagnose subclinical mastitis was evaluated. Per farm only a small number of dairy cows were included in the study. This was due to the fact that only a few cows started their dry period when the IRT pictures were taken. Even though, no cows were selected, due to the sample size there is a possible selection bias.

IRT has been used in experimental settings to detect IMI. After intramammary inoculation of one of the hind quarters with an *E. coli* strain, IRT pictures were taken from the caudal view of the udder (Pezeshki et al., 2011). The infected quarter showed a significantly higher temperature ($p = 0.04$) in comparison to the control quarter. The quarter temperature range found lay in between 29 °C and 34 °C. In the case of clinical mastitis, a change of 2-3 °C in temperature of the udder skin was found. However, the increase in udder temperature measured with the IRT camera occurred after local signs of clinical mastitis were already visible, such as swelling of the udder and changes in milk appearance (flakes, color). Indicating that the IRT camera may not be successful as an early detection method. A similar study by Hovinen et al., (2008), in which the left front quarter was inoculated with an *E. coli* strain showed that the IRT camera was effective in detecting a 1-1.5 °C temperature change in the infected quarters. Yet, in this case local signs of mastitis, such as changes in the milk appearance and a rise in the SCC were visible before the increase in temperature could be measured with the IRT camera.

The effectiveness of IRT in detecting subclinical mastitis has been researched by Polat et al., (2010). Interrelationship between the SCC, CMT and the udder temperature were examined. Two different cut-off values for the SCC were used to diagnose a quarter with subclinical mastitis, above 200,000 cells/ml milk and above 400,000 cells/ml milk. The sensitivity and specificity for IRT to detect subclinical mastitis with a cut-off of 400,000 cells/ml milk were 95.6 and 93.6. With a cut-off of 200,000 cells/ml milk the sensitivity was 83.47 and the specificity was 100. Quarters with a SCC above 400,000 cells/ml milk had a 2.35 °C greater udder surface temperature than the quarters with a SCC below 400,000 cells/mL milk. A significant association has been found between the SCC and the udder temperature. This suggests that IRT could be used for the detection of subclinical mastitis.

In the present study, IRT pictures were taken within 24 hours of drying off. No cut-off values were used for the SCC on cow- or quarter level because no significant association was found between the quarter temperature measured with IRT and the SCC on cow- and quarter level. This means that a higher SCC was not equal to a higher udder quarter temperature. Furthermore, no significant association has been found between the positive bacterial culture and the quarter temperature measured with IRT. Another reason no cut-off values were used was due to the sample size, only a small number of dairy cows had a SCC above 200.000 cells/ml of milk.
No dairy cows with clinical mastitis were included in this study. As shown by Pezeshki et al. (2011) and Hovinen et al. (2008) an increase in quarter temperature was correlated with an increase in the SCC, but only after clinical signs of mastitis were present. In addition to this factor, only 12 of the 53 dairy cows included in this study had a SCC on cow level above 200,000 cells/ml milk. This means that the fact no significant association has been found between the SCC on cow level and the quarter temperature may partially be due to the sample size. 123 of the 212 quarters had an SCC higher than 200,000 cells/ml. In comparison to the number of dairy cows with a SCC on cow level, the number of dairy cows with a high SCC on quarter level is higher. This means the analysis with the SCC on quarter level has more magnitude than the analysis with the SCC on cow level. However, no significant association has been found between the SCC on quarter level and the measured quarter temperature with IRT. This means even in the quarter with a high SCC on quarter level, this did not necessary result in a higher quarter temperature.

Possible environmental and cow level variables have been studied in their relationship with the quarter temperature measured with IRT. To reduce bias, the quarters diagnosed with IMI through a positive bacterial culture were removed from the data set for these analyses. For instance, the environmental temperature seems to have a significant association with the quarter temperature. Placing the environmental temperature into the multivariate model means that any difference in environmental temperature between different days or different dairy farms was taken into account, making the circumstances in which the IRT pictures are taken and analyzed as equal as possible.

The sample size for the analysis of the association between the mean and maximum udder temperature and the presence of intramammary infection and subclinical mastitis was lower than expected since in 37 of the milk samples no clear bacterial analysis could be performed. In these milk samples a mixed flora was found. This may have affected the outcome of the statistical analysis.

As expected, a significant association was found between the presence of an intramammary infection and the SCC on quarter level. No significant association was found between the presence of an intramammary infection and the SCC on cow level. Despite this, the association is almost significant which shows that there still is a relationship between the SCC on cow level and the presence of subclinical mastitis. The reason significance was found with the presence of subclinical mastitis and the SCC on quarter level and not with the SCC on cow level may be argued by the fact that the SCC on cow level is not measured at the same time or with the same milk sample the bacterial culture is taken from. The maximum period of time between the measurement of the SCC on cow level and the moment of drying off (when the bacterial culture is performed) is six weeks. A present intramammary infection present when the SCC on cow level was performed may already have been resolved. In the period between the SCC on cow level was measured and drying off an IMI may also have occurred.

In comparison to the study by Pezeshki et al. (2011), in the present study the range of temperatures measured is a lot wider. The present study also shows that the environmental temperature affects the quarter temperature measured with IRT. Another factor resulting in a wider range of temperature may be the amount of heat radiating from the hind leg, using a different analyzing method, described in the next part, gives a smaller difference in measured quarter temperatures. This means it may also result in a smaller range in quarter temperatures overall.

Difference between day 1 and day 2

In most cases the IRT pictures were taken the day after the start of the dry period. No difference in quarter temperature was found between the day of drying off and the day after. This means that even though the IRT pictures of the present study were in most cases taken the day after drying off, this should not result in large quarter temperature changes and does not interfere with the outcome of the study.

The IRT pictures were analyzed using a polygon measurement tool, following the research by (Metzner et al., 2014). Metzner et al., researched different geometric tools for analysis that were available for the analysis of thermographic pictures. IRT pictures were taken of udders with acute mastitis and healthy udders. Significant changes in udder temperature were measured best using the polygon measurement tool and measuring the maximum udder temperatur. Note, only the hind quarters were analyzed in this study.

To ensure that the polygon measurement tool was the best way to analyze the IRT pictures, two more analyzing methods were researched. First, following the research by (Hovinen et al., 2008), only the region above the teat end was analyzed. This region was found to have the smallest normal variation in temperature of the udder quarter. In the present study, no significant difference was found in the mean and maximum udder quarter temperature between the day of drying off and the day after. Secondly, the polygon measurement tool was used but the region of the udder in contact with the hind leg and the mammary groove were not analyzed. Metzner et al. (2014) showed that a larger difference in the standard deviation was found if the region next to the hind leg and the mammary groove were included into the measurement using the polygon tool, because these are the regions where the highest temperatures could be measured (hind leg only). The present study confirms that the location of the maximum temperature in most cases is found in the mammary groove or next to the hind leg. This effect is most likely the result of heat emission from the adjacent body surfaces. Using the polygon measurement tool with only the middle of the quarter, no significant difference was found between the quarter temperatures measured at the day of drying off and the day after. Because in the present study no significant association was found between the mean and maximum quarter temperature and the SCC and/or presence of intramammary infection, no conclusion can be made if one of these other analyzing methods is better than the polygon measurement of the entire quarter in diagnosing subclinical mastitis. Nonetheless, both the region above the teat end and the middle of the quarter show less influence by temperature radiating from the hind leg which may influence the mean and maximum temperature measured using IRT. If further research was performed, it would be recommended to analyze the IRT picture with using these methods in healthy cows to determine the best analyzing method.

Further research

After the application of IRT pictures to diagnose subclinical mastitis in the present study, certain changes should be made in further research. Most importantly, a larger sample size with more dairy cows with a higher SCC on cow and quarter level should be included into the study. Like in this study, multiple dairy farms should be included and environmental factors like the environmental temperature, humidity and milk production should be noted. Using IRT data, a cow specific normal thermographic profile should be created in the dairy cows with a low SCC and negative bacterial culture by taking IRT pictures over a period of time before drying off. Comparing the thermographic profile of the dairy cows with a low SCC and high SCC may result in visible changes in quarter temperature.

Conclusion

In conclusion, the present study shows no effect of the IRT camera to diagnose subclinical mastitis before drying off. No significant association has been found between the mean and maximum temperature measured with the IRT camera and the SCC on quarter and cow level and the presence of subclinical mastitis. This means that use of an IRT camera in practice as a quarter level diagnostics would not be useful and there is no added value on top of the SCC on cow level. The present study did show a difference in significance in the use of SCC on cow level and quarter level in relation to IMI. Further research is recommended to determine the importance of diagnostics on quarter level.

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