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Mastitis detection in sheep by infrared thermography

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A B S T R A C T

This study aims to evaluate the use of an infrared thermograph for mastitis diagnosis in sheep. Thirty-seven Santa Inês ewes were evaluated weekly through infrared images obtained with thermograph FLIR System Series-i®. Milk was collected for somatic cell count and milk compound level determination. The clinical mastitis group had the highest fat and protein level, as well as the lowest lactose level. The udder temperatures were higher for subclinical mastitis group. The udder temperature data was able to correctly classify the animals into the mastitis groups and the canonical analysis showed that these temperatures clearly differentiated the subclinical mastitis groups from the others. Therefore, this study showed that udder infrared temperatures can be used as diagnostic method to mastitis in sheep.

The most discussed animal disease in the world is probably mastitis, which is responsible for large economic losses to dairy, beef, sheep and goat producers (Freitas, 2005). In general, economic losses are estimated based on lower production and discarded milk due to clinical mastitis. However, subclinical mastitis causes great losses, due to the continuous action of microorganisms in the mucosa, during one or various lactations periods, which provokes a progressive loss of secretor epithelium, reducing milk production and impairing offspring growth (Sommerhauser et al., 2003; Pugh, 2005).

Udder infection may produce an increase in temperature at this organ. Recently, there has been speculation regarding the application of infrared thermography (IRT) to obtain udder surface temperature and, consequently, use it as tool for mastitis diagnosis (Berry et al., 2003). Subclinical mastitis in sheep represents up to 95% of mastitis cases and the absence of clinical signals turns it difficult to detect and treat. The use of IRT for early mastitis detection would be advantageous (Berry et al., 2003). So, this study aims to evaluate the use of infrared thermograph as a tool to help in sheep mastitis diagnosis, notably subclinical mastitis.

Thirty-seven Santa Inês (hair breed) ewes were evaluated weekly throughout their lactation (approximately 12 weeks), except during the perinatal period. The ewes were aged between 2 and 5 years. Lambs were maintained with their dams during the experimental period. All animals stayed at pasture during the day and, at night were fed with corn silage (*ad libitum*) and 250 g/animal/day of concentrate supplementation.

Udder thermal images (Fig. 1) were taken with the ewe in supine position at 1.5 m distance from the udder skin using the FLIR System Série-i® thermograph adjusted to an emittance coefficient equal to 0.97. The temperatures at front, intermediate and rear positions of each half of udder was taken, as well as the temperature at teats, using the point tool of QuickReport® software. The total half udder temperature was measured using the area tool. The photos were taken once a week always at 9 h aiming to avoid differences due to a circadian fluctuation as demonstrated by Berry et al. (2003).

The experiment was carried out in University of Brasilia, Brazil, during the winter (dry period) which is characterized by low temperatures at night (next to 5 °C) and high temperatures during the day (next to 30 °C). The mean air temperature, air humidity and wind speed at the measurement time was 9.3 °C, 88.3% and 1 m/s, respectively. As demonstrated by Berry et al. (2003), it is uncertain how udder temperatures will behave in other seasons, which can be important when comparing this study with others.

Milk samples were obtained immediately after the thermal image collection. The lambs were separated from their mothers 4 hours beforehand and the milk samples were collected through hand milking using a single flask for each udder half. If dirt was attached to the udder, it was cleaned with warm water and these animals were put at the end of the line aiming to regularize superficial temperature, with each animal having at least 10 min before to be examined again. Milk underwent a California Mastitis Test

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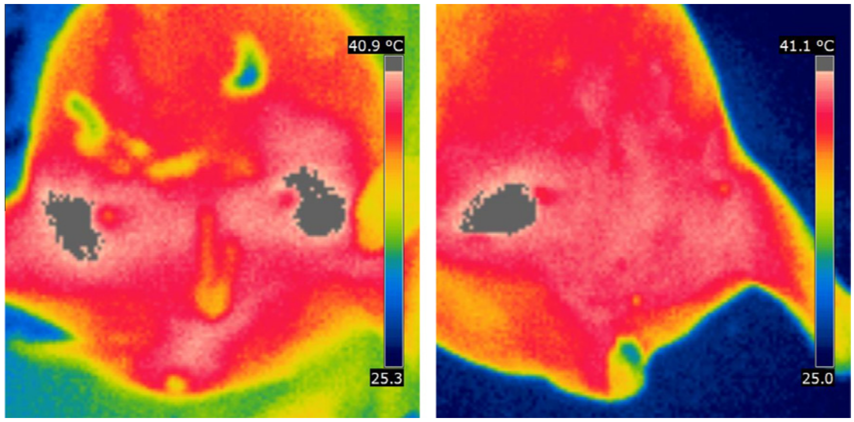

Fig. 1. Thermographic images of the udder of ewes in this study, showing an udder with mastitis at both sides (left) and an udder with mastitis at one side (right).

Table 1
Superficial udder temperatures and milk components from ewes classified as Health, Subclinical and Clinical mastitis.

		Healthy	Subclinical mastitis	Clinical mastitis
Total udder (°C)	Max	38.56[b]	39.02[a]	38.4[b]
	Min	33.56[b]	33.79[a]	33.35[b]
	Mean	36.06[ns]	36.3[ns]	35.89[ns]
Left side (°C)	Front	42.21[ns]	37.33[ns]	36.45[ns]
	Intermediate	37.19[b]	37.50[a]	36.90[b]
	Rear	37.17[a]	37.42[b]	36.69[c]
	Mean	37.01[b]	37.48[a]	36.60[b]
Right side (°C)	Front	36.85[b,a]	37.14[a]	36.53[b]
	Intermediate	37.22[b]	37.72[a]	36.84[b]
	Rear	37.16[a]	37.50[a]	36.71[b]
	Mean	37.08[b]	37.44[a]	36.74[b]
TCL (%)	Fat	5.68[b]	6.17[b]	7.12[a]
	Protein	5.40[b]	5.17[b]	5.82[a]
	Lactose	5.00[a]	4.91[a]	4.60[b]
	FFDM	11.25[a]	10.99[b]	11.29[a]
	TDM	17.43[b]	16.67[c]	18.41[a]
SCC (×1000 cells/mL)		167.1[b]	540.7[b]	2693.2[a]

[a,b,c]Different letters at the same row indicate statistical difference through Tukey test ($P < 0.05$). ns: not significant; FFDM: fat free dry matter; TDM: total dry matter; MCL: milk compounds level; SCC: somatic cell count; Max.: maximum; Min.: minimum.

(CMT) and somatic cell count (SCC), using the Fossomatic 5000 Basic® equipment. Fat, protein, lactose, total dry matter (TDM), and fat free dry matter (FFDM) levels were determined using Milkoscan 4000® (Foss Electric A/S. Hillerod, Denmark).

The animals were divided in three groups each week according to results of clinical and milk analyses. The first group (Healthy) was composed by ewes without responsive mammal lymph nodes or udder rigidiness, negative CMT, and with SCC below 250,000 cells/mL (42% of observations). The second group (Subclinical Mastitis) represented ewes with responsive mammal lymph nodes, mild udder rigidness, mildly positive CMT and SCC between 250,000 and 500,000 cells/mL (23%). The third group (Clinical Mastitis) was composed by ewes with mammal lymph nodes responsiveness, rigid udders, positive CMT and SCC above 500,000 cells/mL (35%). Ewes were classified weekly and could change group depending on their status as above.

The result of udder infrared temperatures, milk compounds and lactation stage of ewe were submitted to analyses of variance using the GLM procedure in SAS® 9.2. Factor, canonical and discriminant analyses were also carried out using the FACTOR, CANCORR and DISCRIM procedures, respectively.

In general, the Subclinical mastitis group had higher udder infrared temperatures than the Healthy and Clinical mastitis group (Table 1). This may have occurred as the Clinical mastitis group had an intense and chronic inflammatory process, which led to lower temperatures than animals in an acute inflammatory process as seen in the Subclinical mastitis group. The mastitis inflammatory process begins through a dilatation of blood vessels, which increase the blood flow to infected udder, increasing the temperature at this point. In a chronic stage, the edema decreases the blood flow to organ and, hence, the superficial temperature decreases (Jones and Plassmann, 2002; McGavin and Zachary, 2007). Moreover, in an advanced stage of the infection, the affected portions of the organ can lose their function and, consequently, reduce their metabolism and blood flow, since 98% of udder blood flow in lactating sheep is through lobulo-alveolar tissue, which is directly responsible to attend the nutritional requirements of this tissue (Thompson, 1980).

Hovinen et al. (2008), evaluating the use of infrared thermal image to diagnosis of mastitis induced by lipopolysaccharides of *Escherichia coli* in cows, demonstrated that inflammatory process can result in detectable increase in superficial temperature; other situations with more edema may not result in a higher superficial temperature. Agreeing with this, Paulrud et al. (2005) demonstrated an increase in teat IRT due to an acute lesion (overmilking) and concluded that this is a useful method to evaluate teat tissue integrity.

Subclinical mastitis group showed a lower fat free dry matter (FFDM) and total dry matter (TDM) than other groups. Similar studies showed variable responses to TDM and FFDM between healthy and sick animals (Zafalon et al., 2000; Zafalon and Filho, 2007). Probably, TDM and FFDM levels had different patterns depending on the stage and intensity of the disease. Kitchen (1981) states that udder infection lead to a decrease in milk fat percentage, which can be explained by the decrease in fat synthesis due to injury on secretory cells. Nevertheless, Leitner et al. (2003) evaluated that fat percentage in healthy glands was lower than infected glands. Burriel (1997) found that the intrammamary infection by *Staphylococcus* coagulase-negative caused an increase in fat and protein level. Indeed, it seems that the increase in milk compounds proportion can occur due to a great reduction in milk volume, despite the lower synthesis of these compounds (Leitner et al., 2003; Burriel, 1997; Schultz, 1977). In a study with inoculation of *Staphylococcus aureus*, Santos et al. (2007) found that the infection led to a significant fat reduction, initially, and, after, led to a decrease in overall secretion of milk compounds.

In the present study, Clinical mastitis group had lower lactose level in milk, showing that the secretory tissue damage may lead to a decrease in lactose biosyntheses as found by Nunes et al. (2007). The clinical mastitis group also had higher fat and protein level, which can be explained by the reduction in milk production. Still, the higher protein level in milk can be related to a high level of serum proteins due to immune response (Leitner et al., 2003).

The first factor showed that protein, fat, and TDM early in the lactation are accompanied by higher udder temperatures and higher lactose content. Therefore, due to the lactation progress, udder temperatures were lower, which is expected due to a metabolism reduction. The second factor showed that higher udder temperatures was related to lower milk lactose level and high DM, SCC, fat and protein levels. These can be an indicative that the acute inflammation (demonstrated by higher temperatures) causes damage in lactose secretory cells, diminishing the lactose level in milk. Still, the concentrations of others milk compounds had an increase due to a decrease in milk production as discussed previously.

The discriminant analyses using IRT data correctly classified animals in their mastitis status in approximately 73% (Healthy, Subclinical and Clinical), while using the milk compounds data correctly classified 85% of healthy animals into their group. TDM and milk lactose level were the variables that differentiated between mastitis groups through stepwise selection procedure. So, these are good indicators of udder health status in sheep. The infrared temperature at center position of udder was the most important variable to differentiate between the groups, therefore this can be the best IRT point looking for mastitis diagnosis.

The canonical representation of ewes showed clearly that, when milk components are used, the Subclinical mastitis ewes are placed between the healthy animals and those with clinical mastitis. Still, when udder infrared temperatures are used, the Subclinical mastitis ewes are separated from the other two, showing the usefulness of IRT to differentiate the Subclinical mastitis group from the others.

The results demonstrate that infrared udder temperatures can be a good auxiliary diagnostic method to mastitis in sheep, principally to subclinical mastitis. Therefore, thermography is a promissory technique for subclinical mastitis diagnosis in sheep. Nevertheless, further investigations are needed to improve the diagnosis accuracy by this method.

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