Computers and Electronics in Agriculture 127 (2016) 413–417

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Original papers

Use of thermographic images to detect external parasite load in cattle

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A R T I C L E I N F O

Article history:
Received 27 January 2016
Received in revised form 3 July 2016
Accepted 5 July 2016

Keywords:
Automatic counting software
Horn flies
Infrared thermography
Ticks

A B S T R A C T

The aim of this study was to compare standard visual methods with infrared thermography combined with a program that contrasted points in the image analysis for automatic counting of ectoparasites (ticks and horn flies) in cattle under pasture conditions. Twenty Holstein cows, 10 Devon cows and 10 Braford bulls were used. External parasites were visually counted and thermographic images taken with a FLIR® T300 camera. Images were processed in Quick report® and parasites also were counted using ImageJ®. Ectoparasites were cooler than the body of the animals in the thermographic images and therefore were easily highlighted by Quick report® program. In the case of ticks, they were especially visible when gravid female ticks were engorged but difficult to observe when hair was long. Horn flies stood out perfectly on thermographic images and were seen down to 3 mm in length. The correlation between visual and thermographic combined with a program counts method was 0.82 for horn flies. The correlation between these methods was 0.32 for the ticks on the lateral of the animal and 0.84 for the ticks on the distal of the animal. The regression between visual and thermographic measures for horn fly was highly significant for the three breeds. The use of thermographic images combined with a program for automatic counting was a useful tool and more accurate than the standard visual methods for counting external ectoparsites in cattle.

1. Introduction

Ticks (*Boophilus microplus*) and horn flies (*Haematobia irritans*) are the most important ectoparasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses both through the direct effects of blood sucking and indirectly as vectors of pathogens and toxins (Jongejan and Uilenberg, 1994). Also, as discussed by Guglielmone et al. (1999), these parasites are reported to reduce hide quality in cattle.

Brazil has the largest commercial cattle herd in the world with more than 180 million animals (Machado et al., 2010) and wastes approximately 390 million kg of meat/year (US$ 600 million) and 4 billion liters of milk/year (US$ 700 million) due to tick burdens (Martinez et al., 2006). In addition, infestation with ticks causes major losses in leather quality. Regarding the quantification of losses for horn flies, Honer and Gomes (1992) calculated that an animal with an annual infestation average of 500 flies could lose 40 kg live weight, which reflects a total loss of meat in central Brazil of 1.4 million tons/year if all animals were parasitized by the fly.

Control methods are necessary, but infestation needs to be quantified. Quick, efficient and accurate counting of ectoparasites that infest farm animals are still poorly studied. The method used for counting ticks is based on the count of engorged females sized between 4 and 8 mm in diameter by palpation on one side of the animal and the value is extrapolated to the other side of multiplying by two (Villares, 1941; Wharton and Utech, 1970). For counting horn flies, the methodology employed is visual counting one side of the animal and multiplied by two to obtain the total value (Guglielmone et al., 2000). The animals are restrained, and in some cases, with the need for greater precision, the animals are filmed and then the footage is used to count the number of flies present on the animal (Lima et al., 2002).

Infrared thermography (IRT) is a remote sensing technique that relies on thermal reaction emitted by all bodies at nonzero temperatures (Holst, 2000). Infrared thermography enables the collection of an image representing the surface of the animal (Souza et al., 2008). Thus, as reported in a recent review by Luzi et al. (2013) infrared thermography is a valuable tool for the determination of physiological events. Ectoparasites do not generally have their

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http://dx.doi.org/10.1016/j.compag.2016.07.002
0168-1699/

own temperature. How their body temperature is regulated by the environment and generally there are temperature differences between the surface of the parasite and the surface of the animal. Thus we can hypothesize that the parasite stand out in the thermographic pictures regardless of the color of the coat of the animal. Additionally, the use of thermographic photographs coupled with a program that contains contrasted points in the images can be a useful tool and more accurate for counting ectoparsites then the standard visual counting method.

Therefore, the aim of this study was to compare standard visual methods with thermography combined with a program for image analysis for automatic counting of ectoparasites (ticks and horn flies) in cattle under pasture conditions.

2. Material and methods

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the University of Brasilia, number 22773/2012.

The study was carried out on a commercial farm in Rio Grande do Sul State, south Brazil at 29°45′ latitude South and 57°05′ longitude West, during the Summer period (December to March of 2014). The climate is classified as Cfa, according to Köppen-Geiger. Twenty Holstein cows, 10 Devon cows and 10 Braford bulls aged approximately 24 months were used in this experiment. The animals had not been submitted to insecticidal treatment, with free access to water and mineral supplement on a natural pasture, which basically consisted of grasses including as *Paspalum notatum* and other species belonging to the genus *Paspalum, Aristida pallens* and *Andropogon lateralis*.

The animals were gathered monthly in a corral between 7:00 and 10:00 a.m. and restrained individually in a chute, held between 2 and 3 min until they were quiet and to let the flies sit on them. Two methods of counting ticks and horn flies were used. One method (visual method) trained observers counted the number of visible flies on the right surface of the animal and multiplied by two to obtained the total value (Guglielmone et al., 2000). After that, the number of engorged tick females greater than 4 mm in diameter were counted by palpation on the right surface of the animal and the value was multiplied by two to determine a total of ticks on the host (Wharton and Utech, 1970). The second counting method (thermographic images combined with a program for automatic counting) was performed immediately after the observer count of each animal. The Infrared thermography images were obtained with a thermographic camera FLIR T300, which had a precision of ±2 °C and infrared resolution of 320 × 240 pixels. Emissivity (ε) was set at 0.98 based on the recommendation of the camera manufacturer for biological tissues. This camera takes simultaneous digital and infrared images so the accuracy of visual and infrared methods could be compared. The camera was calibrated for the temperature and relative humidity before to collect images. A 1 cm metal object was placed on the side of the animal as a size reference. The Infrared thermography images were taken perpendicularly at a 1 m distance from a whole right side and from regions where the greatest number of ectoparasites were present (scrotum, flank, fore-back and face). The focus was regulated to take a best quality of each image. Three images and counts were taken per animal. Images were first processed in Quick report® which was provided with the camera. This was used to alter the color palette from "rainbow" (camera default) to "inverted grey" to improved image definition for counting parasites and then the program ImageJ® (http://imagej.nih.gov/ij/) was used to automatically count the external parasites.

Meteorological data were collected from a weather station located 10 km from the place of the experimental area and included the mean maximum temperature, the mean minimum temperature, and the rainfall level.

Statistical analyzes were performed using the Statistical Analysis System® package (v.9.3, SAS Inc, Cary, NC, USA). The associations between visual methods and thermographic images combined with a program for automatic counting of ectoparasites were investigated using correlation (PROC CORR) and regression analysis (PROC REG).

3. Results

The temperatures at the beginning and at the end of the experiment were 23.5 and 22.8 °C respectively, and the mean relative humidity of the experimental period was 68%.

Changing the pallete color from "rainbow" (camera default) to "inverted grey" improved image definition for counting parasites. This allowed identifying the region of the scrotum and flank affected by individual ticks more clearly and visibly (Fig. 1).

Ectoparasites were cooler than the body of the animals in the thermographic images and were easily highlighted by Quick Report® program. In the case of ticks, they were especially visible when gravid female ticks were engorged (Fig. 1). When small, they were frequently hidden under the animal hair, but in other regions, such as the face, they were easily seen (Fig. 2).

No significant differences were seen between breeds of cattle for the ability to count ticks using this method with a correlation of 0.32 between visible and thermographic methods for ticks on the side of the animal and 0.84 on the hind end of the animal. These difference were mainly due to long hair on the side.

In the case of horn flies, which is a completely exogenous parasite, flying over the coat of the animal, they stood out perfectly on thermographic images and were seen down to 3 mm in length (Fig. 3).

The correlation between visual and thermographic images for horn fly counts was 0.82 and increased to 0.90 when ImageJ® was adjusted to remove small dirt particles. Repeatability of measurements on the same animal was 0.87. No significant differences were seen between breeds of cattle for the ability to count flies using this method.

The regression between visual and thermographic measures for horn fly was highly significant for the three breeds (Fig. 4).

4. Discussion

Cattle kept under field conditions tend to be parasitized simultaneously by different species of ectoparasites (Costa et al., 2014). As ectoparasites cause high economic losses to livestock, and adversely affect livestock hosts in several ways, the animals ectoparasites infestation need to be correctly quantified for the adoption of effective control. Several studies have shown that *Bos indicus* is more resistant to ectoparasites than *Bos taurus* and the introduction of *B. indicus* genes into the herd promotes an increase in the resistance of animals against ectoparasites without compromising production (Oliveira and Alencar, 1990; Wambura et al., 1998). In this study, there was no difference between the tick and horn flies loads among the breeds, most likely due to the low parasite loads and/or to the animals' parasite-resistance.

In addition, our results demonstrated that a standard visual method and thermographic images combined with a program for automatic counting of ectoparasites were highly correlated for horn flies (0.82) in cattle regardless the breed evaluated. Ticks had a lower correlation which depended on hair cover.

The visual counting methods used to quantify ectoparasites infestation is very open to errors. In the case of horn flies, these errors are mainly related to the dispersive behavior of the flies.