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Infrared thermography as a non-invasive method for detecting fear-related responses of cattle to handling procedures

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Abstract

Two experiments were conducted to determine whether maximum eye temperature, measured using infrared thermography (IRT), could be a non-invasive technique for detecting responses of cattle to handling procedures. Experiment one used six crossbred heifers randomly assigned to two groups in a crossover design and subjected to i) being hit with a plastic tube on the rump and ii) being startled by the sudden waving of a plastic bag. Experiment two used 32 crossbred bulls randomly assigned to three treatments: i) control, restraint only; ii) electric prod, two brief applications of an electric prod or, iii) startled, as in experiment one, accompanied by shouting. Exit speed (m s^{-1}) was recorded on release from the restraint. Maximum eye temperature was recorded continuously pre- and post-treatment. In experiment one, eye temperature dropped rapidly between 20 and 40 s following both treatments and returned to baseline between 60 and 80 s following hitting and between 100 and 120 s following startling. In experiment two, eye temperature dropped between 0 and 20 s, following both treatments, and returned to baseline by 180 s, following startling plus shouting, but did not return to baseline for five minutes following electric prod. Exit speed tended to be faster following the electric prod. In conclusion, IRT detected responses that were due possibly to fear and/or pain associated with the procedures and may therefore be a useful, non-invasive method for assessing aversiveness of handling practices to cattle.

Keywords: *animal welfare, cattle, eye temperature, fear, handling, infrared thermography*

Introduction

The understanding and identification of animal handling techniques that can cause fear and pain in animals on commercial farms is of importance, both from an animal welfare perspective, and from the point of view of livestock industry economics. The increasing size of modern commercial farms, time constraints and labour-saving technologies, such as robotic milking systems, reduce the contact animals have with humans and increasingly most of the contact that they do have is negative (Rushen *et al* 1999b) (eg restraint, transport, veterinary procedures). Negative attitudes of stockpeople towards animals on commercial farms are largely responsible for high levels of fear towards humans that impact on animal welfare and productivity (Hemsworth & Coleman 1998; Hemsworth 2007). Aversive cattle handling by stockpeople (eg shouting, quick, unpredictable movements) and the type of handling aids used (eg flags, sticks and prods) can lead to a fear of humans which not only has a detrimental effect on animal welfare but also leads to reduced animal production and an increased risk of injury to both animal and handler (Hemsworth & Coleman 1998; Rushen *et al* 1999b; Hemsworth 2003). Hitting and aversive handling of dairy cows has been shown to reduce milk yield (Breuer *et al* 1997; Rushen *et al* 1999a) and increase heart rate (Rushen *et al* 1999a), weight loss (Breuer *et al* 1997) and lameness (Chesterton *et al* 1989; Breuer *et al* 1997). In addition, excessive use of handling aids may, in fact, hinder rather than facilitate movement of cattle (Rickenbacker 1959). All of these factors incur major economic costs to not only the farm but livestock industries as a whole.

A lack of available tools exist to measure fear and pain responses of cattle, therefore few studies have examined responses to different handling techniques and the use of specific handling aids. Researchers have used a combination of behavioural and physiological responses to measure fear. Some behavioural responses that have been used to measure fear in cattle include flight distance (Fisher *et al* 2000; Breuer *et al* 2003; Kilgour *et al* 2006), time to approach a handler (de Passillé *et al* 1996;

Rushen *et al* 1998), vigilance (Welp *et al* 2004), vocalisations (Grandin 2001) and open-field tests (Kilgour 1975). Caution is required when interpreting behavioural responses, for example, in an open-field test, increased activity as a measure of fearfulness may be influenced by other factors, such as novelty, social motivation, familiarity with the environment and handlers, curiosity and general activity or exploration (Rushen 2000). In addition, behavioural responses may not be as effective at indicating the severity of a noxious experience compared to physiological indicators, such as the hypothalamic-pituitary-adrenal (HPA) axis or sympathetic activity (Mellor *et al* 2000).

Few studies have examined the physiological responses, such as HPA axis or sympathetic activity, which may be related to cattle's fear of human handling. The acute physiological response to fear has two main components. Firstly, the rapid-onset, short-lived, sympathetically-mediated, catecholamine response, which activates the 'fight or flight' reaction and, secondly, the slower-onset, longer duration, cortisol response, mediated by the HPA system (Mellor *et al* 2002). Changes in plasma cortisol concentrations in response to stress during painful husbandry procedures in cattle have been well established (Stafford & Mellor 2005). However, fewer tools are available to measure the acute sympathetic response to fear. During the 'fight or flight' reaction, heart rate increases and blood flow is redirected away from the extremities to organs and musculature (vasoconstriction). A rapid drop in eye temperature, measured using infrared thermography (IRT), observed following disbudding of calves without local anaesthetic, may be a sympathetically-mediated response via vasoconstriction (Stewart *et al* 2008). Nakayama *et al* (2005) found a drop in nasal temperature, measured using IRT, of rhesus monkeys (*Macaca mulatta*) after exposure to a threatening person. Eye temperature, measured using IRT, has been used as a non-invasive tool for measuring stress in other species (Stewart *et al* 2005) and increased in response to velvet antler removal in elk (*Cervus elaphus canadensis*) (Cook *et al* 2005), jugular catheterisation of dairy cows (Stewart *et al* 2007) and a fright in humans (Levine *et al* 2001).

The objective of the present study was to determine whether eye temperature, measured by IRT, could be a non-invasive technique for detecting responses of cattle to various handling procedures. The handling procedures studied were a sudden unpredictable movement (startling using a plastic bag), with or without shouting, and different handling aids commonly used on commercial farms to move cattle (hit with a plastic tube or a shock with an electric prod).

Materials and methods

The study consisted of two experiments. The protocol and conduct of both experiments were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand and the Lacombe Animal Care Committee, Alberta, Canada.

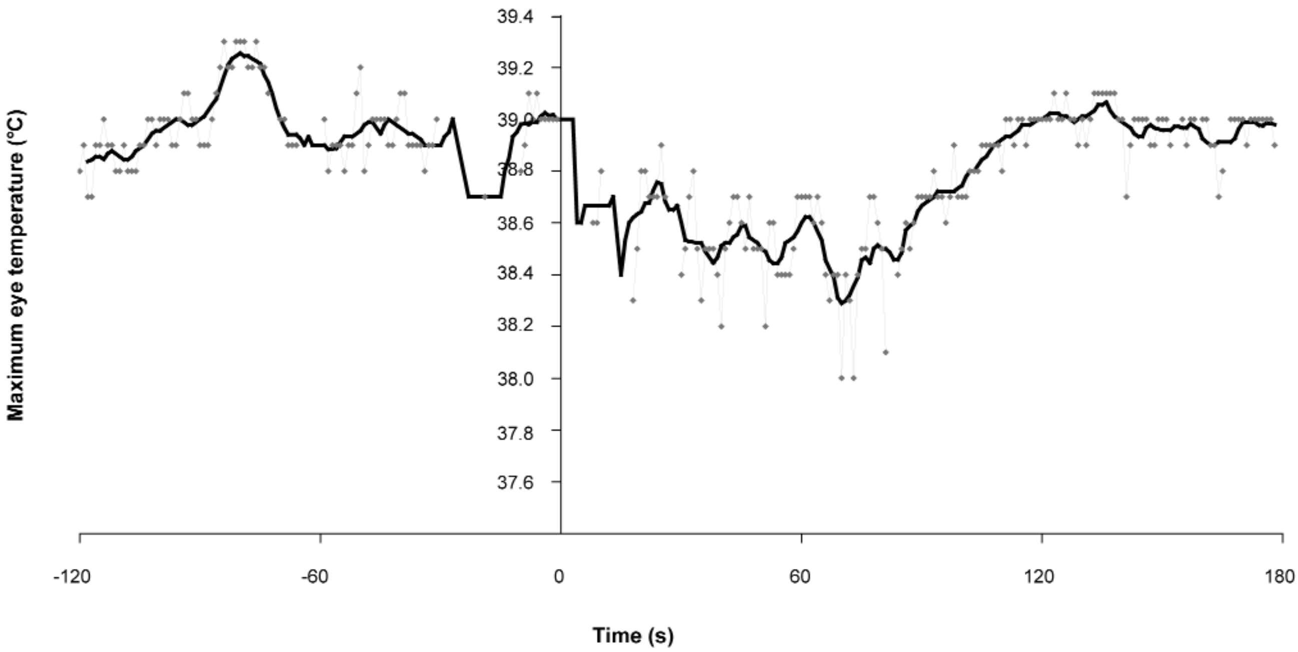
Experiment one

The first experiment took place at AgResearch Ltd, New Zealand. Six, non-pregnant Hereford × Friesian heifers (16 months old), weighing approximately 400 kg, were used. Two months prior to the start of the trial, all animals were halter-trained and brought into covered yards where the trial took place for 2 h per day (four days per week), to habituate them to being handled, and loosely tethered in the facility. Animals were randomly allocated into two groups and received two treatments in a crossover design. One treatment, hitting, consisted of three brief slaps on the rump with a 1 m length of plastic tubing. The other handling treatment, startling, consisted of two brief, sudden shakes of a plastic bag in front of the animal's head. Two animals were given the startling treatment each day for three days and then given the hitting treatment over three following days. All treatments were carried out by the same operator and sampling took place at the same time each day, between 0900 and 1200h, to reduce any circadian effects. Each animal was randomly selected for treatment and was brought into the yard and tethered loosely by a rope halter along with two companion animals. Infrared images of the eye region were collected at a consistent distance (approximately 0.5 m) and angle (90°) from the left side of the animal using an infrared camera (ThermaCam S60, FLIR Systems AB, Danderyd, Sweden). The camera was set to calculate and display the value and position of the maximum temperature within a circular area of analysis on each frame. The area of analysis was restricted to the medial, posterior, palpebral border of the lower eyelid and the lacrimal caruncle (Stewart *et al* 2008). From ten minutes prior to treatment until ten minutes after, the infrared camera was connected to a digital handycam (Sony DCR-TRV355E, Sony, Japan) to enable recording of each video frame. The maximum temperature imprinted on each frame was retrieved by converting the video into digital files and examining each file, frame-by-frame (25 frames per second) using The Observer, version 5.0 software (Noldus Information Technology, Wageningen, The Netherlands) over the period from two minutes prior to treatment until three minutes after. The maximum temperature was averaged for each second and used for analysis. Ambient temperature and relative humidity in the yard were recorded and entered into the infrared camera to ensure calibration for atmospheric conditions.

Experiment two

The second experiment was carried out at Lacombe Research Centre, Canada. This study used thirty-two crossbred bulls, averaging 350 kg, randomly assigned to three treatment groups: i) control, restraint only (n = 13); ii) electric prod, two brief 1 s applications of an electric cattle prod (9000 V, Hot Shot HS2000, Hot-Shot Products Co Inc, Minnesota, USA) applied to the rump area (n = 10) or iii) startling plus shouting, two brief, sudden shakes of a plastic bag in front of the animals head accompanied by a loud shout (n = 9). Treatments were randomised and balanced across three test days and carried out by the same operator.

Figure 1



Maximum eye temperature (°C) for one calf before and after startling in experiment one. 0 seconds indicates the time of treatment. The solid black line represents a 19 s moving average and the grey line indicates the raw data for this individual.

Sampling occurred at the same time each day, between 0800 and 1200h, to reduce the effects of any circadian influence. Each animal was brought into a restraining chute situated inside a barn, and allowed a five-minute rest period, post capture, followed by a 40-minute sampling period prior to being released. Infrared images of the eye region were recorded continuously using a video cassette recorder (JVC HR-S9400U, Wayne, New Jersey, USA) connected to an infrared camera situated 2 m from the left side of the animal, at a right angle, for 20 minute pre- and post-treatment. As each animal exited the restraining chute it interrupted an infrared beam and sensor unit set up 1 m from, and perpendicular to, the head gate. This event started a timing system that was stopped as the animal passed a second infrared beam and sensor unit 2 m from the first. The time taken to travel between the two sensors and the distance travelled (2 m) were used to calculate a chute exit speed (m s^{-1}). Image analysis software (ThermaCam Researcher 2.7, FLIR Systems AB, Danderyd, Sweden) was used to determine the maximum temperature (°C) within the area of the medial, posterior, palpebral border of the lower eyelid and the lacrimal caruncle every 1–3 s during a five-minute pre-treatment and five-minute post-treatment period. Ambient temperature and relative humidity inside the barn were also recorded and entered into the infrared camera to allow calibration for atmospheric conditions.

Statistical analysis

Mean (± SE) eye temperature was expressed as the difference from baseline (ie average over 20 s pre-treatment) at consecutive 20-s blocks, post-treatment. A one-way ANOVA was then used to compare differences between treatments and a Student's *t*-test was used to compare differences at various periods post-treatment from baseline. A one-way ANOVA was also used to test for differences in exit speed between treatments.

Results

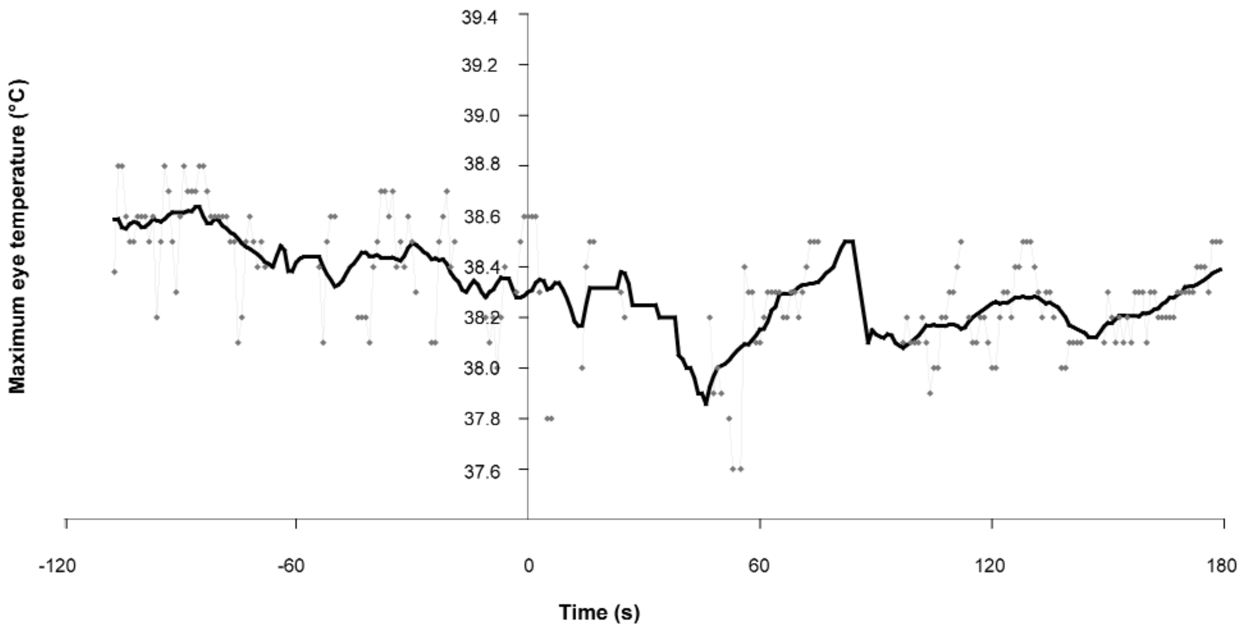
Experiment one

Eye temperature dropped rapidly between 20 to 40 s by 0.23 (± 0.08)°C; ($P < 0.05$) and 0.32 (± 0.05)°C; ($P < 0.01$) after hitting and startling, respectively. Eye temperature remained lower than baseline from 40 to 60 s following startling only (–0.22 (± 0.07)°C; ($P < 0.05$). Eye temperature returned to baseline levels between 60 and 80 s following hitting and between 100 and 120 s following startling (see Figures 1 and 2).

Experiment two

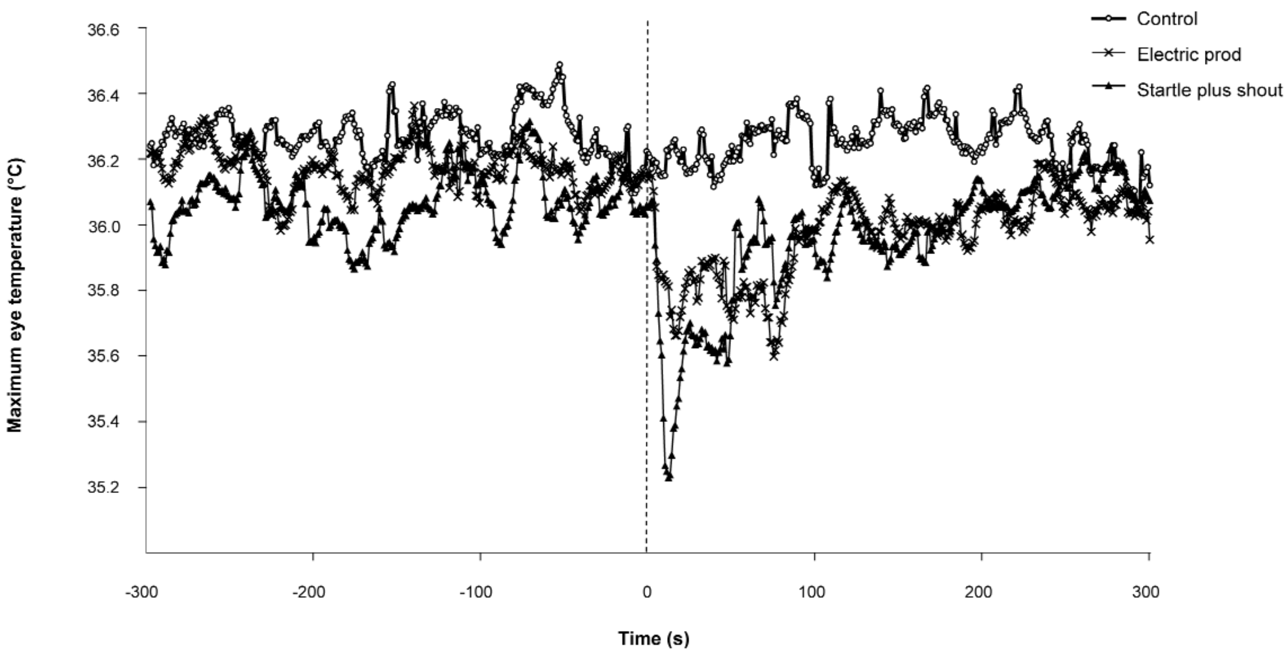
Eye temperature dropped rapidly from 0 to 20 s following both treatments (electric prod; –0.42 [± 0.12]°C; $P < 0.01$ and startling plus shouting; –0.57 [± 0.12]°C; $P < 0.001$) and was still lower than baseline in both treatments from

Figure 2



Maximum eye temperature (°C) for one calf before and after the hitting treatment in experiment one. 0 seconds indicates the time of treatment. The solid black line represents a 19 s moving average and the grey line indicates the raw data for this individual.

Figure 3



Maximum eye temperature (°C) 5 min pre- and 5 min post-treatment, using a 9 s moving average, following control (○, n = 13), electric prod (x, n = 10) and startle plus shout (▲, n = 9) in experiment two. The dashed line (0 seconds) indicates the time of treatment.

20 to 40 s post-treatment (electric prod; –0.32 [± 0.11]°C, $P < 0.01$ and startling plus shouting; –0.43 [± 0.12]°C, $P < 0.01$). At 80 s post-treatment, eye temperature was lower than baseline ($P < 0.05$) in the electric prod treatment only. Following startling plus shouting, eye temperature had returned to baseline levels by 180 s, however, following the electric prod, eye temperature did not reach baseline levels again during the entire five-minute post-treatment period (Figure 3). Eye temperature did not change following the control treatment. Compared to

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controls, eye temperature was lower for both the electric prod and startling plus shouting treatments from 0 to 20 s ($P < 0.05$). From 20 to 40 s, only the startling plus shouting treatment had a lower eye temperature ($P < 0.05$) than controls. There were no significant treatment differences between the electric prod and startling plus shouting. Exit speed from the chute tended to be faster following the electric prod (2.2 [± 0.3] m s^{-1}) compared to the control (1.7 [± 0.3] m s^{-1}; $P = 0.188$) or the startling plus shouting (1.9 [± 0.3] m s^{-1}; $P = 0.434$) treatment.

Discussion

This study has shown that eye temperature, measured using IRT, can detect acute responses that may be due to the fear and/or pain associated with handling of cattle. Eye temperature dropped rapidly following all aversive treatments. The magnitude and duration of the drop in eye temperature was consistent with previous studies (Nakayama *et al* 2005; Stewart *et al* 2008). For example, following disbudding of calves without local anaesthetic, eye temperature dropped rapidly and was lower (–0.27°C) within the first five minutes post-treatment than baseline (Stewart *et al* 2008). Similarly, the nasal temperature of monkeys dropped by 0.2°C within 10–30 s (mean duration of the decrease 220–280 s) following exposure to a threatening person (Nakayama *et al* 2005).

The drop in eye temperature may be a sympathetically-mediated response. Following disbudding of calves without local anaesthetic, the drop in eye temperature was accompanied by a decrease in heart-rate variability parameters that reflect a change in sympatho-vagal balance and may indicate an increase in sympathetic activity (eg the ratio of the low- [LF] to high-frequency [HF] power) (Stewart *et al* 2008). Blessing (2003) reported that fear and anxiety resulting from perception of a threat or possible dangerous event, with or without actually experiencing the actual physical attack or pain, can cause sympathetically-mediated cutaneous vasoconstriction. When vasoconstriction occurs, blood flow to the peripheral capillary vessels is reduced and, as a consequence, skin temperature decreases (Vianna & Carrive 2005). Vianna and Carrive (2005) used IRT to measure stress responses of rats that were fear-conditioned by exposure to footshocks, and found a decrease in tail and paw temperature (–5.3 and –7.5°C, respectively) due to cutaneous vasoconstriction. This cooling of the extremities was associated with an increase in freezing immobility, a behavioural response to fear in rats. They suggested that the blood supply to the tail was very sensitive to the level of fear and that the stronger the fear the stronger and longer the duration of the vasoconstriction.

The nature of the stimulus or the level of fear and/or pain that the animals experience may affect the duration of the drop in eye temperature. In experiment one, eye temperature took longer to return to baseline, following startling, compared to hitting. In experiment two, the initial drop in eye temperature was not significantly different following startling plus shouting, compared to the electric prod; however, there was a longer lasting response and a tendency for exit speed from the chute to be faster following the electric prod. This could be interpreted as a difference in the degree of aversiveness between the two treatments. However, the aversiveness of an experience may also be due to its novelty or suddenness. Desire *et al* (2006) found that suddenness rather than unfamiliarity was responsible for a greater increase in heart rate of sheep exposed to a rapid, compared to a slow, appearance of a scarf, and suggested that the startle response is dependent on the suddenness of the event. The significance and interpretation of the responses found in the present study requires further investigation to determine the potential of IRT to measure the relative aversiveness of different handling procedures.

Shouting has been shown to be aversive to cattle (Waynert *et al* 1999; Pajor *et al* 2000). Pajor *et al* (2000) found that cows took longer and required more force to be moved down a race following repeated treatments with an electric prod or being shouted at compared to being hit with an open hand or having their tail twisted. However, Pajor *et al* (2003) found that cows in a Y-maze showed no preference between shouting or hitting. Shouting has also been shown to increase the heart rate and movement of beef cattle in a restraining chute (Waynert *et al* 1999). The use of electric prods has been associated with vocalisations of cattle at commercial slaughter plants indicating that the devices are aversive to cattle (Grandin 2001). Lefcourt *et al* (1986) gave cows a range of electric shocks from 2.5 to 12.5 mA and found that as mA increased, heart rate increased, cows became more agitated and some responded violently. It is difficult to compare the present results to those of other studies comparing the aversiveness of handling aids because of possible inconsistencies in the type of negative handling and the force and way in which they are applied.

While there was no increase in eye temperature in the present study, other studies have shown that eye temperature can increase in response to fear or pain (Levine *et al* 2001; Cook *et al* 2005; Stewart *et al* 2007). In addition, Stewart *et al* (2008) found that after an inital drop, eye temperature increased following disbudding with or without local anaesthetic. It is possible that the treatments in the present study produced insufficient stimulation to cause an increase in eye temperature. The mechanism for this increase is yet to be determined, however, there is evidence that it is not driven by changes due to heat, physical activity, increased HPA activity or local inflammatory processes (Stewart *et al* 2008). Due to the short time frame of the drop in eye temperature, studies that have only reported increases in eye temperature may have failed to detect an initial drop in eye temperature because sampling occurred too infrequently.

Several factors may influence the eye temperature response, such as breed, temperament or experience with human contact. The present study was not designed to compare these effects on eye temperature, however, they warrant further investigation. To minimise the potential for confounding autonomic stimulation, animals should be habituated to the specific sampling conditions wherever possible. Other factors, such as the angle and distance of the camera from the animal also need to be taken into account when using IRT. However, it is still possible to achieve consistent measures of eye temperature in an outdoor, unrestricted situation. For example, an infrared camera located at a water trough was used to collect images automatically when animals visited the trough to drink (Stewart *et al* 2005; Schaefer *et al* 2007). In the present study, angle and distance were kept consistent and it is unlikely that these factors had any influence on the results. In addition, the drop in eye temperature could not be attributed to evaporative heat loss caused by moisture in the eye. Evaporative heat loss depends on the surface area and even a high rate of 4 g m^{-2} trans-epidural water loss corresponds to only 150 W m^{-2} (Mitchell 1977). The small surface area of the eye (0.00001 m^2) translates into less than 0.02 W, which would produce an undetectable (substantially less than 0.1°C) change in eye temperature. See reviews by Stewart *et al* (2005) and McCafferty (2007) for further discussion regarding limitations and recent advances in IRT applications.

It is important to note that IRT has shown promise as a non-invasive measure of sympathetic activity and while to-date, it has been validated during pain and fear responses, its use may be extended to other situations where activity of the autonomic nervous system is changed, such as during pleasure or positive responses (eg provision of resources such as social contact, space or comfortable resting areas). Boissy *et al* (2007) described the potential for heart-rate variability parameters, combined with behavioural responses, for non-invasive monitoring of autonomic activity associated with positive emotions in animals. Similarly, IRT responses to positive situations warrant further investigation and may be complementary to heart-rate variability responses for assessing emotional states in animals.

In summary, this study has shown that eye temperature, measured using IRT, can be used to detect responses to handling procedures in cattle. It is possible that the eye temperature response is due to the fear and/or pain associated with the handling procedures, and is consistent with pain responses to disbudding in calves. The duration of the drop in eye temperature may relate to the level of fear and/or pain an animal is experiencing and may be used to compare the aversiveness of different handling methods. Eye temperature may therefore be a useful addition to behavioural and physiological methods for assessing fear and pain responses to handling of cattle.

Animal welfare implications

IRT has the potential for non-invasive evaluation of fear and/or pain responses in cattle during routine handling practices on commercial farms. It is clear from the results in the present study that the use of electric prods, hitting and shouting are all aversive to cattle during handling and moving, therefore their use on-farm should be monitored and minimised to prevent reduced animal welfare and consequent economic costs to the livestock industries.

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