Infrared thermography is a technique for imaging the temperature of the surface of objects detected by a thermal camera. A thermal camera can absorb infrared radiation radiated from objects and convert it to an image reflecting temperature differences. The image from infrared thermography displays the body surface temperature generated by the combination of heat production inside the body and environmental factors. It allows us to observe systemic changes in body temperature caused by hyperthermia and hypothermia using a body surface as an object, as well as to detect local abnormal changes in skin temperature without touching the body. Thermography has been applied for screening of patients with fever in the medical field [2, 10].

In the cattle clinic, thermography has been used to detect infection with bovine respiratory disease complex [16] and bovine viral diarrhea [17], as well as to evaluate stress condition [4, 19] by measuring the highest temperature of eye circles, which has been reported to have a close correlation with rectum temperature. About a half of cattle experimentally infected with foot-and-mouth disease virus exhibited increased temperature of legs as detected by thermography prior to vesicle formation [15]. Early detection of mastitis [3, 7] and monitoring of hoof condition by thermography in cattle have also been reported [14]. Reproductive applications of thermography include detection of heat [8] and prediction of calving [5].

There has been low reproducibility in thermography of cattle. In human medicine, technical guidelines have been established for examining patients inside an isolated room. The guidelines recommend conducting imaging of patients after acclimation in a room with a constant ambient temperature without wind and sunlight and with movement of patients limited [13]. Although many researchers have reported that various factors affected surface temperature of cattle in a barn, there has been no systematic investigation to prove the exact effects of the factors.

The objective of this study was to investigate the effects of various essential factors of the environment and objects on imaging in order to establish guidelines for cattle thermography.

MATERIALS AND METHODS

Thermographs were taken using an infrared thermal camera (Advanced Thermo TVS-500EX, Nippon Avionics Co., Ltd., Tokyo, Japan) set on a tripod. Imaging was conducted after automatic temperature adjustment of the camera, and image analysis was performed using the Avio Thermography Studio 2007 Version 4.8 software (Nippon Avionics Co., Ltd.).

Investigation of data acquisition conditions: Experiments were conducted inside a room without direct sunlight and wind at 25°C. The imaging frame, a 5 × 5 cm square 0.75 m above the floor, was made by covering a concrete wall with reflective tape. The effects of the following camera settings were examined: distance from the imaging frame (0.5, 1.0, 2.0 and 3.0 m), camera angle with respect to the frame [0 (parallel to the wall)], angled 45 degrees upward and downward with a fixed distance of 0.5 m from the imaging frame] and focal length of camera lens (three different settings with a fixed distance of 0.5 m from the wall), five times for each condition. The focal length was referred to as “long” for setting the focal length at the longest, as “optimum” for...
The optimum focal length and “short” for the shortest focal length. The camera was set at the same height as the frame (0.75 m above the floor) for these investigations.

The effect of camera temperature was examined by imaging the object at 0, 10, 20, 30 and 60 min after turning on the camera, which was set 0.5 m from the object. More than 1 hr after turning on the camera, a heat pack (13 × 9.5 cm, average temperature 53°C, effective for 12 hr) or a cool pack (16 × 13 × 2.5 cm) was placed on the bottom of the camera, and imaging was conducted 30, 60 and 120 min after placing each pack (five times for each time point).

The effect of wind was examined using an electric fan at the highest speed with the airflow directed between the camera and the frame; the examination was performed 10 times each with and without wind. During imaging, the thermal camera was protected from the wind using protection plates.

The effect of humidity was examined using an electric humidifier placed between the imaging frame and the camera. Imaging was conducted 10 times each with and without operation of the humidifier.

**Investigation of object conditions using cattle:** The effects of hair and an ambient temperature were examined using four non-lactating Holstein cattle. Images were taken for both sides of the prescapula. The image object was a 5 × 10 cm area from which the hair was clipped to be used as the “without hair” object, and the same size of area without the hair clipped was used as the “with hair” object (next to the without hair object). All four cattle were acclimated in a room at 15°C for 30 min prior to imaging. Imaging was conducted with a distance from the object area of 1.0 m at the height same as the object area.

Then, the cattle were moved to another room set at 4°C. Imaging was conducted immediately and 30 min after moving cattle and camera into this cool room. Image analysis was conducted for the without hair and with hair areas separately using software, and the average temperature within each area was calculated. Body temperature was determined using a mercury-in-glass thermometer inserted into the rectum.

The relationships between the rectum temperature, ambient temperature, temperatures of both eyeball surfaces and the highest temperatures of each eye circle were examined using six Japanese Black breeding cattle. For this experiment, imaging was conducted inside a barn without wind and direct sunlight during a hot season as well as a cold season when the ambient temperatures were 28 and 9°C, respectively, using the same animals. Imaging was conducted by setting the camera parallel to the object (0.5 m apart). The rectum temperature was measured using a mercury-in-glass thermometer. Image analysis was conducted for a circle within the eyeball, and the average temperature within that circle was expressed as the “eye ball surface temperature”.

Another oval was made about 1 cm outside the eyeball, and the highest temperature within that oval was expressed as “the highest temperature of the eye circle” [19].

The effect of distance after eating on body surface temperature was examined using 7 Japanese Black breeding cattle during a cold season inside a barn without wind and direct sunlight. Imaging was conducted around the left flank, which covers the areas of the rumen with solid, liquid or gas contents. Also imaging was conducted at the center of the right flank. All imaging was conducted at 1.0 m from the objects. Hair within the imaging area (5 × 10 cm) was clipped, and the animals were acclimated to the ambient temperature after hair clipping. The average temperature of each area for imaging was calculated. Imaging was conducted 1, 4 and 7 hr after eating. The ambient temperature was recorded using a recorder (Thermo Recorder TR-72Ui, T&D Corporation, Nagano, Japan), and rectum temperature was measured using a mercury-in-glass thermometer.

Statistical analysis was conducted using one-way analysis of variance followed by the Tukey-Kramer test for the data with normal distributions. For the data without a normal distribution, Kruskal-Wallis one-way analysis of variance was used followed by the Steel-Dwass test for multiparameter comparisons. Comparison between two groups was conducted using the Student’s t-test or Mann-Whitney test. For the seasonal comparison of eyeball surface and the highest eye circle temperatures, the Wilcoxon signed rank sum test was used. The relationship between temperature of the left flank and the time after eating was examined using a repeated measure of one-way analysis of variance. The correlation of two measures and its statistical significance were determined using Pearson’s correlation coefficient for data with a normal distribution. For data without a normal distribution, Spearman’s rank correlation coefficient was applied. All data are expressed as the mean ± standard error.

The use and care of all animals in this study were approved by the Animal Care and Use Committee of Iwate University.

**RESULTS**

**Investigation of imaging conditions: The effect of distance on detected temperature.** The detected temperature was 26.40 ± 0.06°C at a distance of 2.0 m, and it was 26.26 ± 0.05°C at 3.0 m, both of which were significantly (P<0.05) lower than that at 0.5 m (26.64 ± 0.05°C). There were no significant differences between the detected temperatures at the distances of 0.5 and 1.0 m (26.52 ± 0.10°C).

**The effect of camera angle on detected temperature.** The detected temperatures when the camera was set parallel, at an upward angle and at a downward angle with respect to the object frame were 21.80 ± 0.16, 21.68 ± 0.11 and 21.68 ± 0.07°C, respectively. There were no significant differences between these settings.

**The effect of focal length on detected temperature.** The detected temperatures for the long, optimum and short focal length were 26.02 ± 0.07, 26.46 ± 0.05 and 26.92 ± 0.04°C, respectively. Each setting exhibited a significant difference (P<0.05) from the other settings.

There was no significant correlation between the detected temperature and the time after turning on the camera. The detected temperatures were 22.76 ± 0.05, 23.08 ± 0.04, 23.08 ± 0.04 and 23.10 ± 0.10°C for 0, 30, 60 and 120 min after placing a heat pack, respectively. The temperatures were significantly higher (P<0.05) at all time points after 30 min compared with 0 min. The temperatures were 22.68 ±
Fig. 1. The changes in detected body surface temperature with/without hair and effects of ambient temperatures. The image object was a 5 × 10 cm area from which the hair was clipped to be used as the “without hair” object (closed area), and the same size of area without the hair clipped (open area) was used as the “with hair” object (next to the without hair object). Imaging was conducted at 15°C, immediately after moving the cattle to a room with a temperature of 4°C and 30 min after moving the cattle to a room with a temperature of 4°C.

| Location                  | Temperature (°C)            |
|---------------------------|-----------------------------|
| At 15°C                   | 33.9 ± 0.35                 |
| Immediately after moving  | 39.0 ± 0.5                  |
| to 4°C                    | 29.5 ± 0.4                  |
| 30 minutes after moving   | 29.5 ± 0.4                  |
| to 4°C                    | 29.5 ± 0.4                  |

The correlations between ambient, eyeball surface and the highest eye circle temperatures. The temperatures of the eyeball surface were 34.80 ± 0.22°C (n=12) and 31.44 ± 0.27°C, and the temperatures of the highest eye circle were 38.02 ± 0.17 and 36.02 ± 0.08°C for the hot and cold seasons, respectively. The temperatures in cold season were significantly (P<0.01) lower than those in the hot season for both objects.

Regarding the distance between the camera and the object, the longer the distance, the lower the detected temperature. This phenomenon was obvious when the distance was longer than 2.0 m. This is because gases in the air, such as CO₂, absorb more infrared radiation radiated from the object as the distance increases. Using a fixed distance for all imaging is important. In this study, imaging was conducted with a distance from the object of 0.5 m as a general rule. But, in the case of using cattle, imaging was conducted with a distance from the object of 1.0 m, because the cattle will move away if the distance is too short. Depending on the state of the animal object conditions on imaging: The effect of hair and ambient temperature on detected temperature. The changes in detected body surface temperature with/without hair and effects of ambient temperatures are shown in Fig. 1. The detected temperatures with hair and without hair were 28.28 ± 0.36°C (n=8) and 33.9 ± 0.35°C at the mild room temperature, 21.88 ± 0.68 and 31.20 ± 0.32°C immediately after moving to a cool room and 20.86 ± 0.50 and 29.50 ± 0.46°C at 30 min after moving to a cool room. At both mild and cool temperatures, the detected temperature with hair was significantly lower than that without hair (P<0.01).

DISCUSSION

The body surface temperatures of cattle change due to various factors; thus, these factors affect imaging using infrared thermography. These variations make the comparison of images difficult. In order to apply thermography to the cattle clinic, we investigated how various conditions for a thermal camera and objects would affect the results of imaging.
mal, a distance of 1.0 m from the object is better than 0.5 m.

The effect of the angle of the camera was within the range of error reported previously [1]. There were no significant differences when the camera angle was set within 45 degrees with respect to the object. We also found that without using the optimum focal length for the lens of the thermal camera, we could not detect the accurate temperature.

A thermal camera has the ability to correct the imaging temperature automatically based on the difference between the temperature of the camera body and the ambient temperature. In the medical field, warming up the camera prior to imaging is recommended [13]. We found that warming up the camera was not necessary if imaging was conducted in a room with a stable ambient temperature. In our preliminary study without acclimation of the camera temperature and without auto temperature correction by the camera, hot weather, freezing ambient temperature and holding the camera by hand affected the measurements of temperature. In order to demonstrate these phenomena, we attached a heat pack or cool pack to a camera to change the temperature of the camera body intentionally. This caused an increase or decrease in the detected temperature under a stable temperature within the object frame. This suggested that we should avoid imaging immediately after moving a camera to a location with a higher or lower temperature, as well as imaging in very hot or cold weather, in direct sunlight and in the presence of factors that affect the temperature of the camera.

When there was strong air movement generated by an electric fan, the detected temperature was significantly higher. The forced air movements might have warmed the surface of the concrete wall on which the object square was located. This suggested that airflows influence temperature detection when there is a temperature difference between an object and the environment.

The presence of water vapor increased the detected temperature significantly. Water vapor inhibits transmission of infrared radiation from an object. Water vapor radiates infrared, which might have been detected by the thermal camera, causing a reduction in the accuracy of measurements.

The body temperature of livestock animals is influenced by various factors of the environment, such as ambient temperature, humidity, radiation and airflow. In order to maintain a constant body temperature, animals generate heat by metabolism and release heat from the body surface as well as from the respiratory system [11]. The heat release from the animal body depends on temperature gradients between the animal body surface (skin, hair) and the environment. Thus, skin surface temperature and hair temperature are considered interface temperatures determined by body temperature and environmental factors [11]. In the present study, body surface temperatures both with and without hair decreased significantly immediately after moving animals and the camera into a cool room compared with those determined with a mild room temperature. The reduction in temperature was more pronounced for the body surface with hair than that without hair. Environment temperature affected mildly the skin temperature without hair because of the blood circulation adjacent to the skin, whereas environment temperature affected more rapidly the body surface temperature with hair because of no blood circulation in the hair.

The eye circles, especially around the lacrimal glands, contain an abundance of blood capillaries controlled by the autonomic nervous system [18]. Thermography of this area is reported to be useful for early detection of a stress condition [4], pain caused by dehorning [18], infection with bovine respiratory disease complex [16] and bovine viral diarrhea [17] in cattle and the response to intravenous infusion of adrenocorticotropic hormone in horses [19]. The highest temperature of the eye circle was similar to the rectum temperature in mule deer kept in an experimental room at 25–28°C [6]. Ponies kept inside a barn in early fall showed a positive correlation between the highest temperature of the eye circle and rectum temperature [9]. We suspected that the eyeball surface would not reflect body temperature as sensitively as the surface of the lacrimal gland. However, constant moistening with tears by blinking might help the eyeball surface temperature reflect the body temperature. Our investigation did not show correlations between eyeball surface temperature and the rectum temperature or between the highest temperature of the eye circle and the rectum temperature. Perhaps, the eyeball surface and the highest temperature of the eye circle are influenced more by the ambient temperature. Thus, skin surface temperature and hair temperature are considered interface temperatures determined by body temperature and environmental factors [11]. In the present study, body surface temperatures both with and without hair decreased significantly immediately after moving animals and the camera into a cool room compared with those determined with a mild room temperature. The reduction in temperature was more pronounced for the body surface with hair than that without hair. Environment temperature affected mildly the skin temperature without hair because of the blood circulation adjacent to the skin, whereas environment temperature affected more rapidly the body surface temperature with hair because of no blood circulation in the hair.

| Number of cattle | Hot season | Cold season | Both seasons |
|------------------|------------|-------------|--------------|
| Eyeball surface and the highest eye circle temperatures | 0.26 | 0.28 | 0.84** |
| Eyeball surface and rectum temperatures | 0.22 | -0.59 | -0.52* |
| Highest eye circle and rectum temperatures | 0.65* | -0.12 | -0.32 |
| Eyeball surface and ambient temperatures | ND | ND | 0.88** |
| Highest eye circle and ambient temperatures | ND | ND | 0.88** |

**P<0.01; *P<0.05. ND : No data.**
Our present study with thermography of cattle demonstrated that the following conditions should be considered in order to obtain optimum imaging.

1. A constant distance should be used between the camera and object along with an optimum focus setting for the camera within an angle of 45 degrees with respect to the object.
2. Extreme ambient temperatures, such as in a hot summer and cold winter, direct sunlight, high humidity and wind should be avoided.
3. Comparison between thermographs should be conducted only when imaging is conducted at a stable ambient temperature with identical conditions. If this is not achievable, imaging should be performed for a body surface without hair.

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