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Thermal imaging evaluation of the felines paw pad temperature before and after walking: A pilot study

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Abstract: The purposes of this pilot study were to explore the temperature changes of the paw pads in healthy cats before and after a 6-min walking test (6MWT), and to further compare the accuracy of the point and line region of interest (ROI) selection methods of the thermal imaging. Five healthy intact cats were recruited and trained to run on the treadmill, and to keep still during the standing phase on a glass panel when capturing thermographic imaging data. The paw pad temperature was recorded using a thermal camera and then analysed using both the point and line ROI before and after the 6MWT. The symmetry index (SI) between the limbs (left and right) was further calculated. Compared to the baseline, there were significant increases in the paw pad temperature of the forelimbs (point of left $P < 0.001$, point of right $P = 0.048$, point of average $P = 0.002$, line of left $P < 0.001$, line of right $P = 0.007$, line of average $P < 0.001$), hindlimbs (point of left $P < 0.001$, point of right $P < 0.001$, point of average $P < 0.001$, line of left $P < 0.001$, line of right $P < 0.001$, line of average $P < 0.001$), and average value after the 6MWT both using the point and line ROI selection methods. However, there is no temperature difference between the point and line ROI either before or after the 6MWT. In addition, no difference in the SI was found between the baseline and post-test or between the point and line ROI. These results preliminarily indicated that a 6WMT and thermal imaging could be a good combination for further clinical practices to recognise lameness or any other gait disabilities in cats, and both the point and line ROI selection methods can be considered when analysing thermographic data.

Keywords: 6-min walking test; feline; paw pad; temperature; thermography

Biomechanical motion analysis has been used in veterinary studies for many years to assess the motion characteristics and health status of animals (Schnabl and Bockstahler 2015; Song et al. 2019a; Song et al. 2019b; Wang et al. 2019). Various methods have been further developed for quadrupeds (e.g., dogs, cats, horses, and pigs), with kinetic gait data captured by a force plate or a pressure-sensitive plate being the most common (Lascelles et al. 2007; Verdugo et al. 2013; Schnabl and Bockstahler 2015). With these systems, it is much easier to predict and evaluate the severity of the lameness or any other gait disabilities in quadrupeds by collecting the ground reaction forces during movement (Schnabl

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and Bockstahler 2015). However, the accuracy and implementation of the above methods may be challenged when it comes to cats. Normally, it is hard to detect any pain from cats since they have subtle behavioural alternations (Beale 2005; Vainionpaa et al. 2013). On the other hand, poor compliance of cats during a test is also a problem (Vainionpaa et al. 2013). New methods to recognise gait disabilities and pain in cats are, therefore, much needed.

Thermography, also known as thermal imaging, is a non-invasive and safe method that has been applied to detect and monitor temperature changes of the skin's surface in both humans and animals since the last century (Stewart et al. 2007; Vainionpaa et al. 2012). Compared to other tools, such as a physical examination or pain questionnaire, previous research has demonstrated the effectiveness of thermography in detecting gait disabilities and pain in cats (Vainionpaa et al. 2013). Thus, it may serve as a "new method" to solve the above challenges. There are two different methods that have previously been used to select the region of interest (ROI) in thermal imaging, the point ROI and line ROI. Repac et al. (2020) conducted a study on healthy dogs which evaluated the temperature changes of the hindlimb muscles after walking using both ROI methods. They found that the line ROI is more accurate in detecting temperature changes than the point ROI. Nevertheless, more confirmatory studies are warranted in this field.

Similar to the human foot, paw pads are the direct interface between the cats' body and the ground. Interestingly, previous studies have indicated that the footpads of cats play an important role in thermoregulation (Lloyd 1963; Adams 1966). Thus, measuring the temperature differences between the paw pads could help to detect the initial lameness or muscle injuries in cats, especially after a certain amount of exercise. The 6-min walking test (6MWT), which was originally used to assess the cardiopulmonary function in humans, has been widely applied to veterinary medicine to differentiate healthy animals from ones with cardiopulmonary or neuromuscular diseases (Boddy et al. 2004; Swimmer and Rozanski 2011; Acosta et al. 2016; Repac et al. 2020). Due to its simplicity, the effectiveness of recognising a gait disability in animals when combining with thermal imaging is worth investigating more.

Although there are an increasing numbers of thermographic studies in veterinary medicine, no studies, to our knowledge, have investigated the effect of a 6MWT on the paw pad temperature in cats. In addition, the standard method to predict musculoskeletal diseases and pain in cats should be reconsidered. Therefore, this pilot study aimed to evaluate the temperature differences of the paw pads in healthy cats before and after a 6MWT, to compare the accuracy of the thermal imaging between the point ROI and line ROI, which could add references for future veterinary clinical practices that focus on feline orthopaedic disease diagnosis. Based on previous studies, it was hypothesised that a 6MWT will lead to significant increases in the paw pad temperature and there would be a significant difference in the temperature change between the point and line ROI.

MATERIAL AND METHODS

The animals

Five healthy British Shorthair cats, ranging in age from 90 to 120 days and in body weight from 1 kg to 2 kg, were recruited for voluntary participation in this study. All the cats were prospectively checked by the same veterinarian to ensure that they were clinically intact and health. The body weight was measured by an electronic weighing scale. Before the test, an informed consent was collected from the owner, and this study was approved by the Animal Care and Use Ethics Committee of Ningbo University.

Experimental setup

Thermographic imaging data were collected using a thermal camera with resolution of 384 × 288 (Magnity Electronics Co. Ltd., Shanghai, P.R. China). The thermal camera was connected to a laptop and placed 1-m below a glass panel (Figure 1). All the cats were trained to run on the treadmill, and keep still during the standing phase on the glass panel when capturing the thermographic imaging data. During the test day, the owners were required to bring their cats to the lab at least one hour before the test in order to help cats to acclimatise themselves to the new environment. Then, a thermographic imaging baseline was measured. After that, the cats walked at an average walking speed (0.6–0.8 m/s) as previous studies suggested for 6 min on a treadmill with a plastic channel around the cats in case of any interruption (Schnabl and Bockstahler 2015). The thermographic

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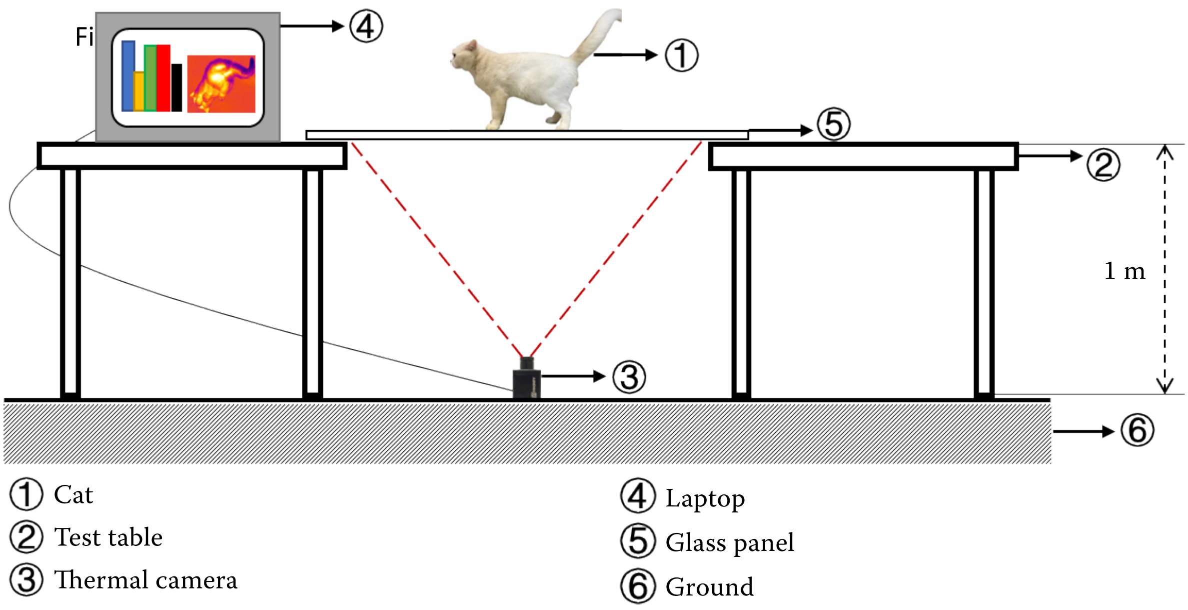

Figure 1. The experimental design used to capture the thermographic imaging data

imaging data were collected again after the 6MWT. At least five valid trials for each cat were collected for further analysis. Toys, food, and encouragement from the owners were used for focusing the attention and cooperation (if necessary). The indoor temperature was controlled at a constant 26 °C and there were no significant movements (e.g., running, jumping) by the cats before the data collection.

Data analysis

The obtained data were analysed by specialised software called ThermoScope v1.2 (Magnity Electronics Co. Ltd., Shanghai, P.R. China). All the data analysis was performed by the same researcher. The superficial temperatures of the paw pads (left forelimb, right forelimb, left hindlimb, and right hindlimb) before and after the test were measured using both the point ROI and line ROI. The centre of the metacarpal pad was selected for the point ROI while the midline was selected for the line ROI (Figure 2).

In addition, in order to set references for future studies investigating the effectiveness of the thermographic imaging on detecting an initial lameness or muscle injuries in cats, a symmetry index (SI) between the limbs (left and right) was further cal-

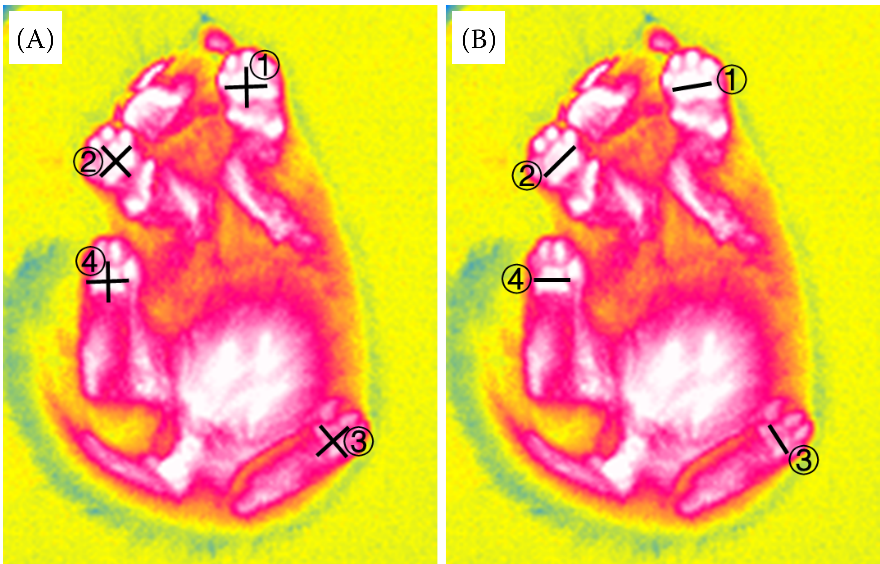

Figure 2. The region of interest (ROI) selection methods of the thermal imaging
(A) Point ROI; (B) line ROI
① left forelimb; ② right forelimb; ③ left hindlimb; ④ right hindlimb

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culated according to the following equation (Voss et al. 2007):

$$SI\ (\%) = (X_R - X_L)/0.5 \times (X_R - X_L) \times 100\% \quad (1)$$

where:
SI – symmetry index;
X_R – mean value of the superficial temperature of the right paw pad;
X_L – mean value of the superficial temperature of the left paw pad.

Statistical analysis

All the statistical analyses were performed in SPSS v23.0 (SPSS Inc., Chicago, IL, USA). A paired-sample *T*-test was applied to compare the differences before and after the 6MWT, while an independent-sample *T*-test was applied to identify the differences between the point and line ROI, regarding the paw pad temperature and the SI. The data were presented as the mean ± SD, and differences were considered significant when $P < 0.05$.

RESULTS

Thermography

As shown in Table 1, there were significant increases in the paw pad temperature of the forelimbs, hindlimbs, and average value after the 6MWT when compared to the baseline using the point ROI. Similarly, the line ROI also demonstrated a significantly greater temperature for the

Table 1. Comparison of the paw pad temperature before and after the 6MWT (unit: °C)

Limb			Pre-test (mean ± SD)	Post-test (mean ± SD)	Difference [95% CI]	*P*-value	Power
Point	forelimb	left	28.72 ± 0.79	29.69 ± 0.43	0.97 ± 0.94 [0.58, 1.36]	< 0.001*	1
		right	29.10 ± 0.65	29.64 ± 0.86	0.54 ± 1.29 [0.01, 1.07]	0.048*	0.89
		average	28.91 ± 0.69	29.67 ± 0.60	0.75 ± 1.05 [0.32, 1.19]	0.002*	0.99
	hindlimb	left	27.78 ± 1.18	29.81 ± 1.81	2.02 ± 2.33 [1.06, 2.98]	< 0.001*	0.99
		right	28.31 ± 1.29	30.59 ± 1.55	2.28 ± 2.46 [1.27, 3.29]	< 0.001*	0.99
		average	28.05 ± 1.19	30.20 ± 1.64	2.15 ± 2.33 [1.19, 3.12]	< 0.001*	0.99
Line	forelimb	left	28.91 ± 0.77	29.93 ± 0.45	1.02 ± 0.92 [0.64, 1.39]	< 0.001*	1
		right	29.25 ± 0.59	29.91 ± 0.75	0.66 ± 1.11 [0.20, 1.12]	0.007*	0.99
		average	29.08 ± 0.67	29.92 ± 0.50	0.84 ± 0.93 [0.45, 1.22]	< 0.001*	0.99
	hindlimb	left	28.06 ± 1.02	30.08 ± 1.78	2.02 ± 2.22 [1.11, 2.94]	< 0.001*	0.99
		right	28.56 ± 1.24	30.78 ± 1.51	2.23 ± 2.41 [1.23, 3.22]	< 0.001*	0.99
		average	28.31 ± 1.08	30.43 ± 1.61	2.13 ± 2.25 [1.20, 3.05]	< 0.001*	0.99

6MWT = 6-min walking test; CI = confidence interval; SD = standard deviation
*Indicates a significant difference before and after the 6MWT

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forelimbs, hindlimbs, and average value. However, regarding the comparison of the temperature differences between the point and line ROI (Table 2), no statistically significant results were found either before or after the 6MWT.

Symmetry

The SI of the paw pad temperature between the left and right paw pad for the forelimb and hindlimb are shown in Tables 3 and 4. There was no signifi-

Table 2. Comparison of the paw pad temperature between the point and line ROI (unit: °C)

Limb			Point (mean ± SD)	Line (mean ± SD)	Difference [95% CI]	*P*-value	Power
Pre-test	forelimb	left	28.72 ± 0.79	28.91 ± 0.77	0.19 ± 0.22 [–0.25, 0.63]	0.387	0.21
		right	29.10 ± 0.65	29.25 ± 0.59	0.15 ± 0.18 [–0.20, 0.50]	0.403	0.21
		average	28.91 ± 0.69	29.08 ± 0.67	0.17 ± 0.19 [–0.22, 0.56]	0.383	0.21
	hindlimb	left	27.78 ± 1.18	28.06 ± 1.02	0.27 ± 0.31 [–0.35, 0.90]	0.337	0.22
		right	28.31 ± 1.29	28.56 ± 1.24	0.25 ± 0.36 [–0.47, 0.97]	0.492	0.16
		average	28.05 ± 1.19	28.31 ± 1.08	0.26 ± 0.32 [–0.39, 0.91]	0.422	0.19
Post-test	forelimb	left	29.69 ± 0.43	29.93 ± 0.45	0.24 ± 0.12 [–0.01, 0.49]	0.064	0.60
		right	29.64 ± 0.86	29.91 ± 0.75	0.27 ± 0.23 [–0.19, 0.73]	0.240	0.31
		average	29.67 ± 0.60	29.92 ± 0.50	0.25 ± 0.16 [–0.06, 0.57]	0.110	0.47
	hindlimb	left	29.81 ± 1.81	30.08 ± 1.78	0.27 ± 0.51 [–0.75, 1.29]	0.594	0.13
		right	30.59 ± 1.55	30.78 ± 1.51	0.20 ± 0.43 [–0.68, 1.07]	0.653	0.11
		average	30.20 ± 1.64	30.43 ± 1.61	0.23 ± 0.46 [–0.69, 1.16]	0.612	0.12

CI = confidence interval; SD = standard deviation; ROI = region of interest

Table 3. Comparison of the SI of the paw pad temperature before and after the 6MWT (unit: %)

Limb		Pre-test (mean ± SD)	Post-test (mean ± SD)	Difference [95% CI]	*P*-value	Power
Point	forelimb	1.57 ± 1.07	1.48 ± 1.58	–0.09 ± 1.94 [–0.89, 0.71]	0.82	0.09
	hindlimb	2.03 ± 2.14	2.89 ± 2.47	0.86 ± 3.54 [–0.60, 2.32]	0.24	0.51
Line	forelimb	1.29 ± 0.89	1.61 ± 1.88	0.32 ± 2.12 [–0.56, 1.19]	0.46	0.27
	hindlimb	2.23 ± 2.07	2.71 ± 2.08	0.48 ± 3.62 [–1.01, 1.98]	0.51	0.23

6MWT = 6-min walking test; CI = confidence interval; SD = standard deviation; SI = symmetry index

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Table 4. Comparison of the SI of the paw pad temperature between the point and line ROI (unit: %)

Limb		Point mean ± SD	Line mean ± SD	Difference [95% CI]	*P*-value	Power
Pre-test	forelimb	1.57 ± 1.07	1.29 ± 0.89	−0.27 ± 0.28 [−0.83, 0.29]	0.33	0.25
	hindlimb	2.03 ± 2.14	2.23 ± 2.07	0.20 ± 0.60 [−1.00, 1.40]	0.74	0.09
Post-test	forelimb	1.48 ± 1.58	1.61 ± 1.88	0.13 ± 0.50 [−0.86, 1.11]	0.79	0.08
	hindlimb	2.89 ± 2.47	2.71 ± 2.08	−0.18 ± 0.65 [−1.49, 1.13]	0.79	0.08

CI = confidence interval; ROI = region of interest; SD = standard deviation; SI = symmetry index

cant difference between the baseline and post-test either using the point ROI or line ROI. In addition, the difference between the point and line ROI also fail to reach a significant level either at baseline or post-test.

DISCUSSION

The purposes of this pilot study were to evaluate the temperature differences of the paw pads in healthy cats before and after a 6MWT using a thermal camera, and to further compare the accuracy of the thermal imaging between the point ROI and line ROI. These results are all valuable to establish basic references for future studies that focus on feline orthopaedic disease diagnosis. We found that there were significant increases in the paw pad temperature of the forelimbs, hindlimbs and average value after the 6MWT both using the point and line ROI selection methods. However, there was no significant difference between the temperature of the point and line ROI. Additionally, no significance differences were found in SI. Consistent with our first hypothesis, there was a significant temperature increase in the paw pad for the forelimb, hindlimb and average value after the 6MWT when compared to the baseline. The higher temperature after the test may partly correspond to the increased thermoregulation effect of the footpad during walking (Lloyd 1963; Adams 1966). However, from a mechanics perspective, there is a chance that the above finding could also be a result of friction as the paw pads are the direct interface between the cats' body and the ground during exercise. Further studies are warranted in order to investigate whether a 6MWT is sufficient enough to induce the thermoregulation effect of a cat's footpad. Our results also found that there was no statistically significant temperature difference in the symmetry between the left and right paw pads of healthy cats before and after the test. Symmetry has been previously used as an important parameter to detect lameness or muscle injuries in animals and humans (Voss et al. 2007; Kim et al. 2011). It was found that an asymmetry of > 1 °C could be an indicator of a potential pathology (Turner 2001). When combining the above research results, it was preliminarily confirmed that a 6MWT and thermal imaging could be a good combination for gait disability recognition in cats.

Regarding the accuracy of the ROI in the thermal imaging, it was found that no statistically significant difference between the point and line ROI either before or after the 6MWT existed, which goes against our second hypothesis. Furthermore, we also compared the difference in the SI between the point and line ROI both before and after the 6MWT, but no significant results were found. Interestingly, previous research investigating the muscle temperature change of healthy dogs after a walking test using a similar ROI selection as the current study also found some different results (Repac et al. 2020). Authors of this study demonstrated that the line ROI would be greater for early pathology detection as there were significant temperature increases in the muscles (e.g., biceps femoris) after walking when using the line ROI, rather than the point ROI. One explanation may contribute to the difference between the above-mentioned study and the current one – since we only investigated the temperature change of a cat's paw pad, the length of the line used in this study would be much shorter than the length

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of the muscle belly chosen by Repac et al. (2020). Consequently, the average temperature of the line ROI measured in this study may be more localised. Nevertheless, more studies are needed for further verification.

Several potential limitations of this study should be mentioned and need to be further addressed. The small sample size and homogeneity of the cats is problematic, which may lead to different results and increase the type II error risk (Song et al. 2019b). Moreover, although the point and line ROI were selected by the same experienced author and assessed by another one, individual variations still existed.

In conclusion, based on the results of this pilot study, we preliminarily demonstrated that a 6MWT and thermal imaging would be effective and easy tool to be used for further clinical practices to recognise lameness or any other gait disabilities in cats. In addition, our results also found that there was no significant difference between the point and line ROI in collecting the paw pad temperature, which indicated that both ROI selection methods would be suitable for analysing thermography results and detecting temperature changes. However, further investigations in this field are much warranted.

Conflict of interest

The authors declare no conflict of interest.

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