Journal of Thermal Biology 106 (2022) 103247

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Evaluation of body surface temperature variations in dogs affected by spinal cord injuries during physiotherapy exercise in a water treadmill

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ARTICLE INFO

Keywords:
Dog
Spinal cord injuries
Infrared thermography
Physiotherapy
Body surface temperature

ABSTRACT

The aim this study was to evaluate variation in body surface temperature (BST) in healthy and spinal cord injured (SCI) dogs, and to outline temperature variation at rest (T0), during (T1) and after (T2) water-treadmill physiotherapy sessions in SCI using infrared thermography (IRT). Sixty-seven dogs of different sex, breed, body weight and age were enrolled: 14 healthy dogs and 53 dogs affected by disc pathologies. The study examined three regions of interest (ROIs): the total image of the spine (IMAGE), the spinal cord area from 1st thoracic vertebra to the last lumbar vertebra (AR01) and the surgery wound or spinal cord lesion area (AR02). Significant BST variations between healthy and SCI were reported in T°max and T°max-min (ΔT) values in IMAGE ($P < 0.05$). In SCI group, AR01 and AR02 assessment showed an increase in temperature ate the sited of the injured area and adjacent body structures. In SCI, a significant effect of water-treadmill exercise in AR01 and AR02 was reported. In fact, both AR01 and AR02 reported higher BST (T°max, T°mean, T°min and ΔT) during the physical exercise (T1), representing the response to physical activity of the spine vascularization, muscles and column contiguous tissues. Furthermore, in T2, the same areas reported a significant lower BST (T°max, T°mean, and ΔT), related to a decrease in tissue inflammation on the target area of the water treadmill physiotherapy. This study highlights how IRT can detect BST variations associated with injured areas. In addition, IRT revealed a positive effect of water-treadmill exercise on the injured spinal cord areas, thus it could be a viable non-invasive and rapid method to support both clinical examination and assessment of the effectiveness of medical treatment in SCI.

1. Introduction

Infrared thermography (IRT) is a no-contact, non-invasive technique that detects surface heat emitted as infrared radiation. Veterinary IRT is a term indicating in vivo digitally imaging an animal with an infrared camera using computer interpretation of thermal maps (Stelletta et al., 2012). Image recognition software is being developed for the objective analysis of the thermal radiation and to generate thermal patterns in the form of a colour map. The colour map reports the temperature differences graphically, highlighting hot and cold spots and showing the thermal distribution of an object or a body surface.

Thermal imaging cameras can produce very sharp images of the of body surface temperature's distribution with an accuracy of 0.08 °C (Luzi et al., 2014a; Turner, 2001). Various trials were performed with different animal species (horse, pig and cows) to assess the validity of the thermographic instrument (Giannetto et al., 2020; Purohit et al., 2012; Rekant et al., 2016; Warriss et al., 2006; Fabbri et al., 2022). The great advantage of IRT in animal research is that measurements can be made without touching the animal and can be made both at close range (<1 m) or at long distances (>1000 m) (Stelletta et al., 2012). The IRT technique presents a number of conditioning factors, which must be consider in order to perform a realistic measurement of BST. The variables that influence BST can be categorized as environmental, subjective or technological factors (Fernández-Cuevas et al., 2015): identification and standardization of these variables are imperative to produce accurate thermal results (Luzi et al., 2014a; Rekant et al., 2016). The effect of the environment on IRT temperature reading can be controlled with the appropriate algorithm and inputs such as distance, humidity, and

Abbreviations: RT, Infrared Thermography; SCI, spinal cord injured dog; ROI, region of investigation; BST, body surface temperature.

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https://doi.org/10.1016/j.jtherbio.2022.103247
Received 12 July 2021; Received in revised form 24 March 2022; Accepted 12 April 2022
Available online 15 April 2022

ambient temperature. (Rizzo et al., 2017b; Vainionpää et al., 2012). The position of the IRT camera in relation to the subject is another important factor which need to be consider during the interpretation of thermographic images (Westermann et al., 2013). Furthermore, multiple studies investigated how individual animal factors can affect IRT results in healthy animals (Berry et al., 2003; Petr and Ivana, 2012; Purohit et al., 2012; Rekant et al., 2016; Turner, 2018). Therefore, BST is reported to be influenced by the coat type, the colour of the skin, the haired and non-haired skin (Kwon and Brundage, 2019; Subedi et al., 2014). Alternatively, it has been noted by two canine studies that haircutting is not necessary to produce accurate thermal patterns in both healthy and injured dogs (Infernuso et al., 2010). Difficulties in the interpretation of thermal patterns arise when using multiple canine breeds, especially when the hair coat is highly variable (Mccafferty, 2007).

The evaluation of BST variations can be used to identify the presence of heat increase body surface areas associated with pathological or physiological conditions, because the abnormal thermal patterns have been associated with body surface areas of inflammation or circulatory impairments (Garcia et al., 2017; Purohit and McCoy, 1980; Redaelli et al., 2014). In terms of both physiological and practical mechanisms, the principal mechanism involved in heat gain or loss is the regulation of the diameter of near-surface blood vessels. In fact, the cutaneous vasodilatation allows heat exchanges with the environment (Mota-Rojas et al., 2021c; Reyes-Sotelo et al., 2021). These regions, described as thermal windows (Casas-Alvarado et al., 2020; Choudhury et al., 2020; Mota-Rojas et al., 2021a), are characterized by a dense network of cutaneous and subcutaneous blood vessels. Variations in blood flow are crucial in the physiological heat exchange between the animal's skin and the environment: these changes occur through vasoconstriction and vasodilatation mechanisms controlled by the sympathetic noradrenergic vasomotor response of the smooth muscles (Ootsuka and Tanaka, 2015). In veterinary medicine, IRT is utilized both to evaluate the physiological status of individual animals (thermoregulation, measurement of food efficiency, lactation processes or diagnosis of pregnancy) (Mota-Rojas et al., 2021b), and to assess pathological disorders and impaired animal welfare (diagnosis of contagious diseases and infections in farm animals, evaluation of stress responses) both in farm and wild animals (Bortolami et al., 2015; Choudhury et al., 2020; Cilulko et al., 2013; Perazzi et al., 2016; Piccione et al., 2013; Schrank et al., 2017). Furthermore, thermographic technique finds various applications in in equine practice (Bogard et al., 2020; Soroko and Howell, 2018; Yarnell et al., 2014), as well as in small animal medicine (Alves et al., 2020; Biondi et al., 2015; Elias et al., 2021; Infernuso et al., 2010; Loughin and Marino, 2007; Luzi et al., 2014b; McGowan et al., 2015; Rekant et al., 2016; Rizzo et al., 2017a).

In small animal medicine, IRT is reported to be sensitive in detecting abnormal thermal patterns related to various clinical conditions, such as neoplasia, ophthalmologic diseases, behaviour and welfare, as well as to identify the localization of orthopaedic and neurological conditions (Elias et al., 2021; Gorre et al., 2020; Kwon and Brundage, 2019; Luzi et al., 2014a; Rekant et al., 2016; Travain et al., 2015). A recent study reported the sensitivity of IRT in localizing the injured area in chondrodistrophic dogs with thoracic disc herniation, comparing with the magnetic resonance imaging results and surgical findings (Grossbard et al., 2014). Furthermore, thermography has been employed in surgical procedures and anaesthesiology in determining and recording vasomotor alterations, and to study the behaviour of C sympathetic fibres (Bruins et al., 2018; Holmes et al., 2003), as well as to evaluate the efficacy of local anaesthetic procedures (Küls et al., 2017). IRT is not limited to detecting pathological changes but, rather, it allows to identify changes in microcirculation caused by the use of drugs, including local anaesthetics. In fact, these studies reported that IRT is a complementary tool for evaluating the functioning of the autonomous nervous system in surgical and post-surgical pain because it represents an objective, accurate technique (Casas-Alvarado et al., 2020).

Physical exercise represents stressful stimulation that can lead to homeostasis disruption with direct impact on animal health status and physical performance: IRT was reported to be accurate in describing the variations of BST in dogs before and after treadmill exercise, comparing with external and core temperature detection devices (Piccione et al., 2012; Rizzo et al., 2017a). Nevertheless, IRT cannot diagnose a specific disease through a colour thermographic image, whereas it permits to visualize the presence of a "thermal anomaly": it is pivotal to contextualize IRT measurements with the physiological phenomenon or the clinical symptoms within the animal's clinical condition.

Preventive medicine represent an important application of IRT technology: in apparently clinical healthy subjects, variations in body surface temperature can represent and localize anatomical or pathological issues that have not yet caused clinical signs (Rekant et al., 2016; Vainionpää et al., 2012). The non-invasiveness of IRT, the relatively low cost, and the absence of adverse effects allow the utilization of this technique as a screening or diagnostic tool potentially in both healthy and pathological subjects. The present study aimed to evaluate the sensitivity of IRT in identifying different thermal patterns of the spinal region in dogs with spinal cord injuries compared with healthy dogs, and to assess the effect of a moderate treadmill exercise session on body surface temperature in SCI. Further studies are required to evaluate the sensitivity of thermal technique to better understand changes in BST in SCI related to the neurologic diseases, the medical treatments and the surgical procedures.

2. Materials and methods

All the dogs were evaluated at the *Veterinary Teaching Hospital* of the Department of Animal Medicine, Production and Health in Legnaro (Padova, Italy) and afterward at the veterinary physiotherapy centre *"Thermal Physiopet"* in Montegrotto Terme (Padova, Italy). Data collection was from March 2017 to April 2018.

3. Sample population and water-treadmill physiotherapy

The study enrolled 67 dogs of different age, sex and breeds: 14 short hair healthy dogs (control group - 5 mixed medium-large-size breed dogs median age 7 years old; 3 male and 2 female dogs and 9 dachshunds, 7 male and 2 female dogs median age 6.5 years old) and 53 dogs with spinal cord injuries (SCI). Among the SCI group, 16 dogs were small-size mixed breed (6 male and 10 female dogs, median age 8 years old), 6 dogs were medium-size mixed breed (2 male and 4 female dogs, median age 6 years old), 9 dogs were large-size mixed breed (5 male and 4 female dogs, median age 7 years old) and 22 dogs were dachshunds (13 male and 9 female dogs, median age 6.5 years old).

Anamnestic data including veterinary clinical report and neurological evaluation were considered for each subjects. Specifically, in the SCI group, 44 dogs had intervertebral disc herniation, 3 dogs had vertebral dislocation and subluxation, 6 dogs were affected by single or multiples intervertebral arthropathies. In dogs with intervertebral disc diseases, spinal lesions were most represented between T11 and L7: 26 dogs reported a thoracic intervertebral disc herniation (17 dogs showed localization between T12-T13 vertebrae; and 9 dogs presented the disc herniation between T11-T12 vertebrae); 5 dogs reported lumbar intervertebral disc herniation (L1-L7). Dogs diagnosed with disc herniation were admitted for neurosurgery more than three weeks before enrolment in the present study. Assessment of surgical patients were performed 3 weeks after the surgical procedure in order to avoid the possible acute alterations due to the inflammation of the surgical wound site.

Intervertebral disc luxations were reported in 3 dogs localized in the T12-T13 spinal region. In addition, 6 dogs presented with single or diffuse spinal arthropathy or localized spinal trauma in both thoracic (T1-T13) and lumbar (L1-L7) regions.

The SCI group was submitted to water treadmill exercise (Hydro

Phisyo™ HP 300)[1], consisting in four walking sessions (1 minute each) of 0.3 m/s for the small breed dogs and 0.6 m/s for the large breed dogs. Water levels were adjusted according to the size of the patient and considering the time since neurosurgery: water level up to the third proximal of the femur or up to the femur condyle. IRT evaluations were performed once at the beginning of the rehabilitation programmes. At this time, all dogs subjected to neurosurgery were not undergoing post-surgical medical treatments.

4. Data collection and infrared thermal imaging camera

Dogs were acclimated without restraint to indoor enclosures with a stable ambient relative humidity and temperature range of 21–25 °C for a minimum of 15 min prior to imaging; the relative humidity levels and the ambient temperature measurements were recorded through an ambient temperature thermometer (AcuRite Digital Weather Station).

Each dogs were submitted to IRT examination at the physiotherapy centre (T0): subjects were placed in standing position without touching the hair or the areas examined. The ROI of the dorsal view extended from the caudal cervical region to the base of the tail. The ROI of the lateral views included the caudal cervical region and the entire lateral surface of the body. The distance from the camera to the dogs was 1 m for dorsal images and 1.5 m for lateral views. The markers were removed after image acquisition. Afterward, the SCI were equipped with a lifebelt harness and started the water treadmill exercise: the second IRT detection was performed during the physiotherapy (T1) while keeping the camera as perpendicular as possible to the dogs' back. At the end of the treadmill exercise in water, the dogs were dried with towels and hair dryer. The third IRT detection (T2) was collected 15 minutes after complete drying to avoid thermal changes related to the environment.

One-hundred-fifteen thermographic measurements were performed through IRT T420 (FLIR®System, Wilsonville, Oregon, USA)[2] and the setting was as follows: temperature range 10 °C–65 °C; emissivity of skin: 0.97; distance between camera and body surface (focus distance): 1.0 m; field of view (FOV): 23°; thermal sensitivity: <0.045 °C; imagine resolution: IR of 76,800 (320 × 240) pixels; PiP and MSX -20 °C–650 °C; accuracy index ± 2% or 2 °C; frame rate of 60 Hz;, thermic colour range *Rainbow*, environmental humidity of 95% (Rizzo et al., 2017a). The detector consisted of a focal plane array (FPA) uncoiled microbolometer with the following specifications: spatial resolution (IFOV) of 1.3 mrad, spectral range between 7.5 and 13 μm accuracy±2 °C. Automatic corrections based on user input were conducted for reflected ambient temperature, distance, relative humidity and atmospheric transmission.

The thermographic technique employed for the examination followed the guide lines reported in literature (Rizzo et al., 2017a; Vainionpää et al., 2012).

Three specific regions of interest (ROI) were defined for SCI: spine total image (IMAGE), the column area from the 1st thoracic to the last lumbar vertebra (AR01) and the surgical wound/spinal cord injury area (AR02) (Fig. 1). Each region of interest of a similar area was selected for all animals and for each time measurement. To reduce the effects of environmental factors on thermographic readings, all images were acquired at the same distance (1 m) from the subject. Camera settings were kept standard as previously described.

IRT measurements were analysed using thermography software (ThermaCAM Researcher Basic 2.8 software, FLIR, Wilsonville, Oregon, USA)[3] for identification of possible ROIs. A maximum (T° max) and minimum (T° min) temperature measurements were recorded for each area, and the difference between maximum and minimum values (ΔT), the mean value (T° mean) were calculated.

5. Statistical analysis

Data were processed using SAS statistical software version 9.4 (SAS Institute Inc., Cary, North Caroline, USA)[4]. The data were tested for normal distribution using the Shapiro-Wilk normality test. A general linear model (GLM) for repeated measures was applied to assess the temperature variation between the healthy and pathologic dogs and applied to assess significant effects of exercise on body surface temperature in SCI in each region of interest, as well as the statistically significant change in temperature values among the considered body surface regions. A PROC MIXED analysis was employed on SCI group and used time (T0-T1-T2), breed, age and sex as fixed factor. The animal was considered as random and repeated effect. Post-hoc least square means comparison was performed using Bonferroni correction. P values < 0.05 were considered statistically significant.

6. Results

Statistical analysis showed no significant differences related to sex, age and breed ($P > 0.05$).

All the 115 IRT performed and their thermic images were analysed through ThermaCAM® Researcher Basic 2.8[c] software: three different areas were identified such as the dog's spine total image (IMAGE), the column area from the 1st thoracic to the last lumbar vertebra (AR01) and the surgery wound/spinal cord lesion area (AR02) (Fig. 1).

Each healthy and SCI was investigated at rest (T0) analysing every identified ROIs, as reported in Table 1. In IMAGE, a statistically significant effect was reported in T° max ($P = 0.011$) and ΔT ($P = 0.025$) difference between healthy and SCI. In AR01, a strong statistical effect ($P < 0.0001$) was reported in T° max and, ΔT between the control and SCI groups, as well as in T° mean ($P = 0.009$). Moreover, in AR02, a statistically significant effect ($P < 0.0001$) was detected in T° max, T° min and T° mean ($P < 0.001$) between normal and pathologic dogs.

In addition, in SCI group the tree different ROIs were investigated at rest (T0), during the water treadmill physiotherapy (T1) and after the exercise (T2), in order to evaluate the possible BST variations related to the physic activity, as reported in Fig. 2 and Table 2 (Fig. 2 and Table 2).

In SCI, a significant effect of water-treadmill exercise in AR01 and AR02 was found (T0-T1-T2). In AR01, T° max showed significant variation during the physical exercise (34.48 °$C_{\_T0}$, 35.17 °$C_{\_T1}$, 34.05 °$C_{\_T2}$) ($P = 0.007$), and T° mean (31.13 °$C_{\_T0}$, 31.92 °$C_{\_T1}$, 31.02 °$C_{\_T2}$) ($P = 0.03$). In AR02, significant effects were reported in T° max (34.50 °$C_{\_T0}$, 35.26 °$C_{\_T1}$, 34.34 °$C_{\_T2}$) ($P = 0.014$) and T° min (32.58 °$C_{\_T0}$, 33.02 °$C_{\_T1}$, 31.32 °$C_{\_T2}$) ($P < 0.0001$), and in T° mean (33.53 °$C_{\_T0}$, 34.14 °$C_{\_T1}$, 32.83 °$C_{\_T2}$) ($P = 0.0005$) and ΔT (1.90 °$C_{\_T0}$, 2.22 °$C_{\_T1}$, 3.03 °$C_{\_T2}$) ($P = 0.0005$).

7. Discussion

IRT lack of invasiveness and high sensitivity allowed to various applications in veterinary medicine (Luzi et al., 2014a). The present study reports on the sensitivity of IRT in detecting differences in BST between healthy dogs and dogs with spinal cord injury, describing higher BST in several ROIs in SCI (Grossbard et al., 2014; Redaelli et al., 2014; Sargent G.R., 2008). Particularly, the statistically significant effect reported in in IMAGE ($_{T°\ max}$ 34.72 °C and $_{\Delta T}$ 15.47 °C), and the significant BST variations reported in AR01 between the control and SCI groups ($_{T°\ max}$ 34.55 °C, $_{\Delta T}$ 6.66 °C, $_{T°\ mean}$ 31.22 °C), demonstrate the IRT feasibility in discriminating between dog's normal and pathological spine areas. In

[1] Hydro Phisyo™ HP 300 water treadmill.

[2] IRT T420 (FLIR®System, Wilsonville, Oregon, USA. Setting: 240 X 360 pixels for imagine IR, PiP, MSX, −20 °C–650 °C, accuracy index ±2% or 2 °C, resolution Pixel IR of 76,800 (320 × 240), thermic sensitivity of <0.045 °C, temperature range 10°C–65 °C, frame rate of 60 Hz, focus distance of 1.0 m, thermic colour range *Rainbow*, environmental humidity of 95%.

[3] ThermaCAM® Researcher Basic 2.8 software.

[4] SAS 9.4 statistical software (SAS Institute, Cary, USA).

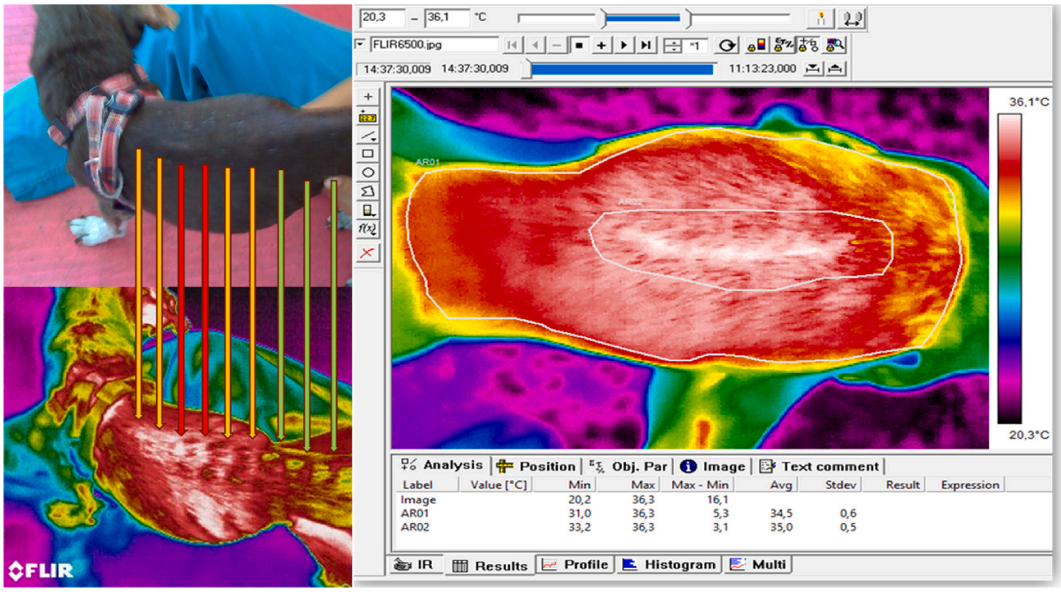

Fig. 1. ThermaCAM® Researcher Basic 2.8 software IRT measurements (°C) of the total image (IMAGE), the column area from the 1st thoracic vertebra (T1) to the last lumbar vertebra (L5) (AR01) and the surgery wound/spinal cord lesion area (AR02).

Table 1
Body surface temperature thermographic detection (°C) of IMAGE, AR01 and AR02 in healthy (Control group) and SCI group at rest (T0), and standard error of the mean (SEM).

IRT measurements		CONTROL GROUP	SCI GROUP	ΔT	SEM	P-Values
IMAGE	T° min	19.52	19.25	0.27	0.6907	0.787
	T° max	33.11	34.72	1.61	0.4265	0.011
	ΔT	13.59	15.47	1.88	0.5674	0.025
	T° mean	26.31	26.99	0.68	0.4990	0.350
AR01	T° min	27.68	27.89	0.21	0.5572	0.790
	T° max	30.94	34.55	3.61	0.4688	<0.0001
	ΔT	3.26	6.66	3.4	0.3264	<0.0001
	T° mean	29.31	31.22	1.91	0.4883	0.009
AR02	T° min	28.86	32.65	3.79	0.4805	<0.0001
	T° max	30.94	34.57	3.63	0.4680	<0.0001
	ΔT	2.08	1.92	0.16	0.2527	0.656
	T° mean	29.90	33.61	3.71	0.4571	<0.0001

addition, in AR02, significantly higher BSTs ($_{T^\circ\ min}$ 32.65 °C, $_{T^\circ\ max}$ 34.57 °C, $_{\Delta T}$ 1.92, $_{T^\circ\ mean}$ 33.61 °C) were reported between normal and pathologic dogs, representing the focal area of hyperthermia in the region above the spine cord injury ("white spot") (Fig. 1). Increased temperatures in the areas of the column and in the region of the surgical wound/lesion can be explained by the presence of an inflammatory process, which leads to increased tissue metabolism and local inflammatory factors (vasodilation or vasoconstriction, oedema, neo-angiogenesis). Specifically, local microcirculation and increased metabolic rate may be clinically related to inflammatory processes, while areas of decreased temperature can be clinically associated with decreased tissue perfusion secondary to a vascular shunt, infarction, or variations in the autonomic nervous system (Church et al., 2009; Eddy et al., 2001; Love, 1980; Naudé et al., 2008; Singer et al., 2019). According to the human medicine literature, the consideration that thee natural increase in the density of surface blood vessels was the cause of the localized pattern of temperature increase was cited (Domán and Illés, 2004; Verheye, 2004; Zhang et al., 1999); whereas, no specific documentation of such a vascular pattern could be found in the horse or the dog. In veterinary medicine, IRT was utilized to evaluate the intestinal vascularization and bowel tissue viability in rats (Malafaia et al., 2008), dogs (Moss et al., 1981; Moss et al., 1981), and pigs (Brooks et al., 2000) before, during, and after experimental induction of ischemia, and results suggest that IRT can be used intraoperative to identify the low perfused areas and compromised intestinal tissue that should be removed to promote successful healing. No statistically significant differences in BST were reported in relation to breed, sex and age. This finding suggest the useful of IRT in discriminating between healthy and SCI despite the possible underlined effects related to subjective characteristics such as morphology, systemic co-morbidities, hormonal function and potential differences in metabolic and/or vascular or inflammatory response (Casas-Alvarado et al., 2020; Jorge et al., 2021).

Furthermore, the overall increases in temperature in SCI group detected in AR02 ($\Delta T_{T^\circ\ min}$ $^{3.79°C,}$ $\Delta T_{T^\circ\ max}$ $^{3.63°C,}$ $\Delta T_{T^\circ\ mean}$ $^{3.71°C}$) define the injured areas, in agreement with the neurological anamnestic data. These findings are consistent with the results of a study conducted in people with cervical intervertebral disc herniation, where IRT not only correctly identified disk herniation but also the location (Zhang et al., 1999). In horses with neurologic injuries, a focal region of hyperthermia representing the affected intervertebral disc space was reported (Purohit et al., 2012; Purohit and McCoy, 1980; Turner, 2001). In addition, the present study confirms the observations reported in a previous veterinary studies which reported that IRT successfully identified the specific abnormal intervertebral disc space in spinal cord injured dogs compared with more sensitive e specific diagnostic technique (nuclear magnetic resonance) and surgical findings (Grossbard et al., 2014).

Finally, our study reports the effect of water treadmill exercise in dogs with spinal cord injuries. Specifically, increases in BST were reported during the physiotherapy exercise on the column area and the spinal regions affected by vertebral or disc pathologies. In SCI group, temperature increased in both AR01 ($\Delta T^{0\text{-}1}$ $_{T^\circ\ max}$ 0.69 °C) and AR02 ($\Delta T^{0\text{-}1}$ $_{T^\circ\ min}$ 0.44 °C, $_{T^\circ\ max}$ 0.32 °C, $_{T^\circ\ mean}$ 0.61 °C) during the water treadmill exercise (T1), represents the response to physical activity of the vasculature of the spine, muscles and contiguous tissues of the column. Physic exercise is associated with physiological changes and alterations of blood flow patterns, leading to body surface temperature variations (Yarnell et al., 2014). The increased blood flow produces a greater distribution of heat, which facilitates heat loss on the body's surface. Surface heating of the dermis is related directly to local dermal microcirculation (vasoconstriction-vasodilatation), which is controlled

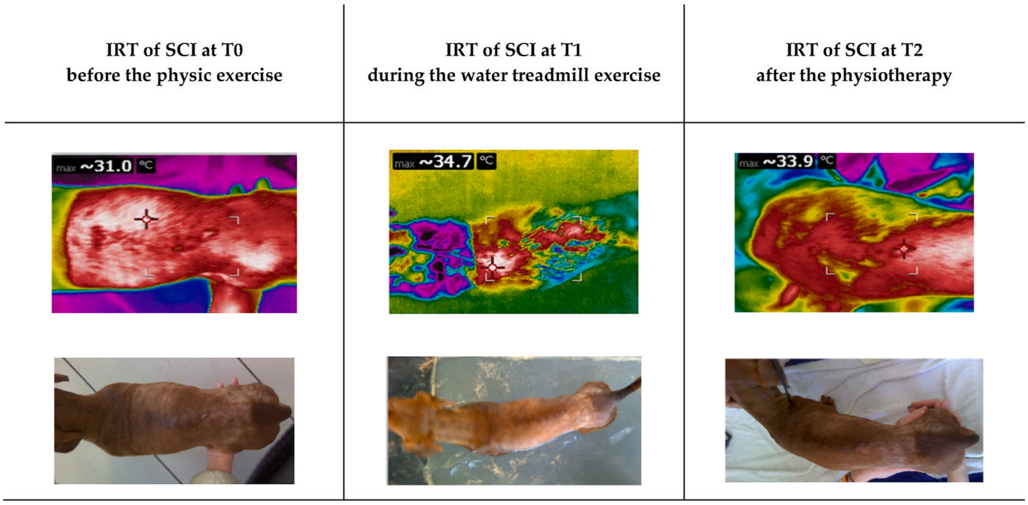

Fig. 2. Body surface temperature thermographic detection (°C) of IMAGE, AR01 and AR02 in SCI group at rest (T0), during the water treadmill physiotherapy (T1) and after the exercise (T2). The red dot (†) represents the maximum temperature point of the spinal injury in the detection area.

Table 2
Body surface temperature thermographic detection (°C) of IMAGE, AR01 and AR02 in SCI group at rest (T0), during the water treadmill physiotherapy (T1) and after the exercise (T2). IRT temperatures mean values and ΔT (T° Max-Min) (°C) and standard error of the mean (SEM). The significant differences within row were represented by (a), and (b).

IRT measurements		SCI GROUP			ΔT			SEM	P-Values
		T0	T1	T2	ΔT_{0-1}	ΔT_{0-2}	ΔT_{1-2}		Time effect
IMAGE	T° min	18.96	20.32	19.83	1.36	0.87	0.49	0.6797	0.0698
	T° max	34.65	34.86	34.95	0.21	0.30	0.09	0.3737	0.6144
	ΔT	15.77	14.56	15.12	1.21	0.65	0.56	0.6115	0.0644
	T° mean	26.81	27.60	27.39	0.79	0.58	0.21	0.4562	0.1355
AR01	T° min	27.80	28.69	28.05	0.89	0.25	0.64	0.5216	0.1723
	T° max	34.48 a	35.17 b	34.05 a	0.69	0.43	1.12	0.3883	0.0071
	ΔT	6.64	6.43	6.03	0.21	0.61	0.40	0.3351	0.4026
	T° mean	31.13 a	31.92 b	31.02 a	0.79	0.11	0.90	0.4281	0.0305
AR02	T° min	32.58 [a]	33.02 [a]	31.32 [b]	0.44	1.26	1.70	0.4108	<0.0001
	T° max	34.50 [a]	35.26 [b]	34.34 [a]	0.76	0.16	0.92	0.3849	0.0148
	ΔT	1.90 [a]	2.22[a]	3.03 [b]	0.32	1.13	0.81	0.2329	0.0005
	T° mean	33.53 [a,b]	34.14 [a]	32.83 [b]	0.61	0.70	1.31	0.3809	0.0005

by the autonomic nervous system (Küls et al., 2017). The most represented physiologic mechanisms involved in heat dissipation are represented by the increase in cardiac output, the respiratory rate and the vasodilation: these mechanisms encourage the heat exchange from core to the cutaneous blood circulation (Priego Quesada et al., 2015). Furthermore, local dermal circulation is directly controlled by the sympathetic nervous system: any conditions which cause variation in the sympathetic tone can alter the cutaneous perfusion, consequently changing the thermographic colours map. The increase in sympathetic tone due to the physic exercise (increase in muscles activity, increase of oxygen-carbon dioxide exchange, increase in energy requirement) can justify the localized area of hyperthermia (white spots) detected during the physiotherapy. The evaluation of the surgical patients was performed 3 weeks after the surgical procedure, in order to avoid possible alterations due to the inflammation of the wound site. Therefore, the statistical analysis revealed no differences between the IRT measurements according to the type of spinal cord injuries and/or the neurosurgery. In SCI, the BST measurements were higher at T1 as mentioned before, while they were significantly decrease after exercise in both AR01 ($\Delta T^{0-2}{}_{T°\ max}$ $^{0.43}$ °C) and AR02 ($\Delta T^{0-2}{}_{T°\ min}$ 1.26 °C, $_{T°\ max}$ 0.16 °C, $_{T°\ mean}$ 0.70 °C). These results would suggest the positive effect of water treadmill physiotherapy, which represents a decrease in tissue inflammation as a response to the rehabilitation and medical treatment on the target area. The decrease in inflammatory factors, such as local oedema, impaired blood circulation and muscles injuries, can be considered the cause of the decrease in BSTs after water treadmill physiotherapy. Although this study cannot provide information on rehabilitation follow up, our results suggest the feasibility of IRT technique to evaluate the effectiveness of the physiotherapy treatment in SCI. Furthermore, the IRT ability to identify BST variations leads this technique to be considered a sensitive tool for preventive assessment of subclinical spinal diseases in dogs. In fact, frequently dogs with spinal cord injuries report several sites of intervertebral disc degeneration, protrusion, bulging that may be not associated with clinical signs. In addition, these subjects may present multiple sites of acute or chronic spinal cord lesions. In this scenario, our findings suggest the possibility of using IRT to improve the clinical assessment of dogs with SCI, as well as IRT can be considered as a screening to select patients for advanced imaging (spinal lesion localization). It is crucial to recognize that various pathological changes can lead to abnormal thermal pattern and therefore IRT may not replace the most sensitive diagnostic tools. Alterations of the IRT colour map may suggest the presence of a spinal disease, related to an inflammatory process, although it cannot provide a specific diagnosis. Advantages of IRT include its non-invasiveness, relatively low cost, lack of exposure to ionizing radiation, lack of anaesthetic requirements, and absence of adverse effects. Our study suggests that IRT would be particularly useful for a preliminary assessment especially in neurological dogs with systemic disorders (cardiovascular, renal, hepatic issues) or dogs with politrauma because it does not require to anesthetize or manipulate the animals. In addition, IRT would be an unbiased method to assess responses to surgical and medical treatments in dogs with spinal cord injuries by reporting changes in microcirculation, inflammatory status and response to surgical or medical treatments, as well as the reaction of spine tissues to the rehabilitation exercises, in the colour heat map. Further investigation would be useful to evaluate the IRT thermal patterns related to biological mechanism and pharmacological or surgical response in dogs with different neurological o vascular conditions, as well as to determine the role of IRT in assessing patient recovery and prognosis.

8. Study limitations

The present study aimed to evaluate the differences in body surface temperature between healthy dogs and dogs with spinal cord injuries. In addition, the study employed IRT to detect BST variations in SCI at rest, during and after a water treadmill physiotherapy session. The SCI undergoing neurosurgery were evaluated after 3 weeks, to avoid alterations due to post-surgical inflammation of wound site. Furthermore, in our study no significant intra-group variations were found in BST among the SCI. No further information on rehabilitation follow up was available. Future studies will be important to investigate the potential of IRT to detect thermal patterns related to the nature of spinal cord injuries and type of surgical or medical treatment.

9. Conclusion

A significant increase in BST was reported between healthy dogs and dogs with spinal cord injuries, representative of the acute or chronic inflammation of the injured spinal cord regions. Furthermore, in the SCI group, IRT showed the positive effects of treadmill exercise in water: the increase in temperature of the target regions during exercise described an increase in tissue metabolism, vascular adaptations and increased muscle activity; conversely, the decrease in BST post-treatment indicated a rational reduction in the inflammatory condition. These finding suggest the possible employment of IRT in monitoring the efficacy of rehabilitative treatments in dogs with SCI. Further studies are needed to describe the sensitivity of IRT used to evaluate different neurosurgical procedures and spinal cord injuries and rehabilitation follow up. Despite the lack of specificity compared to gold standard diagnostic methods (radiology, magnetic resonance), the BST evaluation with IRT could be a viable non-invasive and rapid method to support both clinical examination and assessment of the effectiveness of medical and rehabilitation treatment in dogs with spinal cord injuries.

Author statement

Elisa Mazzotta: Hypothesis generation and experimental design, Organizing and conducting the experiments, Interpreting and analyzing the results, Writing and revising the manuscript.

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Conceptualization, E.M. and E.F.; methodology, E.F., E.T., S.M., C.S.; software, E.F., A.L., C.S.; investigation, A.L., A.V.; resources, E.F., S.M., E.T., A.V., C.S.; data curation, E.M., E.F., A.L.; writing—original draft preparation, E.M.; writing—review and editing, E.M., A.L., E.F.; visualization, E.M., A.L; supervision, E.M.; project administration, E.F.; funding acquisition, E.F. All authors have read and agreed to the published version of the manuscript.

Ethical approval

No approval from Ethics Committee was required. No invasive medical procedures were executed to perform the study. The study was performed with the consent of the animals 'owner during the routinely clinical activity of the Veterinary Teaching Hospital, University of Padua, and of the Thermal Physiopet physiotherapy and rehabilitation activities, from March 2017 to April 2018. Animal care and procedures are in accordance with the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments (National law: D.L. 26/2014).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data statement

Full data from the study are available from the authors on reasonable request.

Declaration of competing interest

The authors declare they do not have any conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

Authors would like thank to the veterinary medical staff of the *Veterinary Teaching Hospital* of the Department of Animal Medicine, Production and Health and the physiotherapy centre *"Thermal Physiopet"* for the clinical and management support.

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