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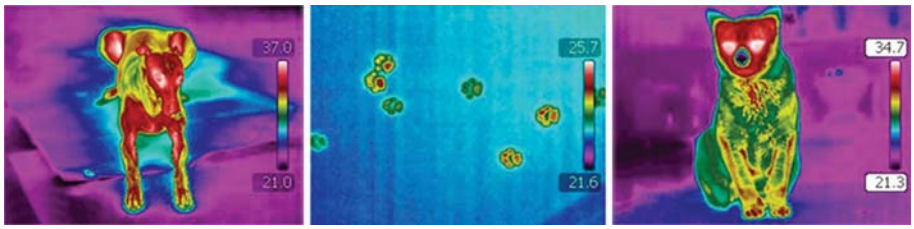
MARI VAINIONPÄÄ

Thermographic Imaging in Cats and Dogs
Usability as a Clinical Method



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Thermographic imaging in cats and dogs
Usability as a clinical method

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in Walter Hall, University EE building, on June 6th 2014, at noon.

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Cover: thermographic images of a racing greyhound lying down, paw prints of a cat and from the front of a cat sitting down taken with the thermal camera FLIR T425

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To my family

Abstract

Thermographic imaging has been studied and used widely in human and equine medicine, but published data from small animal medicine is still lacking. The primary aim of this study was to obtain basic knowledge of the method of thermographic imaging and to map possible areas of its use in companion animals.

To determine the requirements for an optimal thermal camera, three cameras with different resolutions (80 x 80 pixels, 180 x 180 pixels and 320 x 240 pixels) were tested. A total of six thermographic images were taken from the hips of 49 dogs of 26 breeds. Two images were shot with each of the three thermal cameras. Two different persons took the thermographic images with the three cameras. Repeatability between thermographers and interpreters was studied. The usability of basic software for interpreting the thermographic images was examined by having three individuals interpret the thermographic images. The camera with the resolution of 320 x 240 pixels was considered the most suitable for thermographic imaging in dogs and, therefore, the rest of the studies were carried out using this camera.

The impact of physical exercise on canine superficial temperature was studied with 47 racing greyhounds during two race days. Four superficial temperature points from the right and left hind leg were selected (*Tendo calcaneus, Musculus gastrocnemius, Musculus gracilis* and *Musculus biceps femoris portio caudalis*) to compare the changes in superficial temperatures before and after the race. The temperatures differed by 0–4 degrees at each selected point between the right and left legs after the completion of the race. However, no systematic asymmetry was detected between the dogs' left and right side. The superficial temperatures were significantly colder when the ambient temperature was lower.

Cats are known to be challenging subjects in clinical examinations and, therefore, more feline-friendly study methods are required. Thermographic imaging was used to detect temperature differences between both sides of 103 cats and, further, as an indicator of potentially painful processes. Both long-haired (n = 26) and short-haired (n = 77) cats were included. The cats that tolerated manual manipulation were also physically palpated. Owners filled in a questionnaire about the behaviour of their cat and estimated whether the cat was in any pain. The questionnaire responses, the owner's estimation of pain on a scale and thermographic imaging with palpation suggested that thermographic imaging is a potential tool of choice in clinical practice for detecting and screening cats potentially in pain.

Certain drugs used in sedation and anaesthesia affect cardiovascular function. Peripheral temperature changes induced by distinct sedation protocols in dogs (n = 8) were identified by thermographic imaging from the digital and metatarsal foot pads. The obtained foot pad temperatures were compared to the rectal temperature of the same animal. The results indicated that superficial temperature changes caused by certain sedative drugs can be detected and monitored with thermographic imaging.

Our studies suggested that thermographic imaging is a practical clinical method with dogs and cats. However, the effects of physical exercise and medications should be taken into consideration when interpreting thermographic images.

Contents

Abbreviations

ANOVA	analysis of variance
CI	confidence interval
CT	computed tomography
DFT	digital foot pad temperature
DJD	degenerative joint disease
DLC	diamond-like coating
FPA	focal plane array
Hz	hertz, cycle per second
IFOV	instantaneous field of view
iv	intravenously
im	intramuscularly
K	degrees Kelvin
MFT	metatarsal foot pad temperature
MB	medetomidine with butorphanol
MK	MK-467
m	metre
mK	milliKelvin
mm	millimetre
mRad	milliRadian
MRI	magnetic resonance imaging
NETD	noise-equivalent temperature difference
ROI	region of interest
μm	micrometers
°C	degrees Celsius

List of original publications

This thesis is based on the following original articles:

I Vainionpää M, Raekallio M, Tuhkalainen E, Hänninen H, Alhopuro N, Savolainen M, Junnila J, Hielm-Björkman A, Snellman M, Vainio O. Comparison of three thermal cameras with canine hip area thermographic images. *Journal of Veterinary Medical Science*. 2012; 74: 1539-1544.

II Vainionpää M, Tienhaara E-P, Raekallio M, Junnila J, Snellman M, Vainio O. Thermographic imaging of the superficial temperature in racing greyhounds before and after the race. *The Scientific World Journal*. 2012; Article ID 182749, 6 pages, doi: 10.1100/2012/182749.

III Vainionpää MH, Raekallio MR, Junnila JJT, Hielm-Björkman AK, Snellman MPM, Vainio OM. A comparison of thermographic imaging, physical examination and modified questionnaire as an instrument to assess painful conditions in cats. *Journal of Feline Medicine and Surgery*. 2013; 15: 124-131.

IV Vainionpää M, Salla K, Restitutti F, Raekallio M, Junnila J, Snellman M, Vainio O. Thermographic imaging of superficial temperature in dogs sedated with medetomidine and butorphanol with and without MK-467 (L-659'066). *Veterinary Anaesthesia and Analgesia*. 2013; 40: 142-148.

The publications are referred to in the text by their roman numerals (I–IV). The articles have been reprinted with the permission of their copyright holders.

1 Introduction

Thermographic imaging is a method in which a thermographic camera is used to obtain a thermal image of the object. The images, thermograms or thermographic images, are viewed with the camera's display function or computer software. All objects with a temperature of above absolute zero (0 K or -273.15 °C) emit infrared radiation. Infrared radiation is invisible to the human eye, but it is perceived as heat (Figure 1). The infrared spectrum commonly detected with thermographic imaging is between 7.5 and 13 µm.

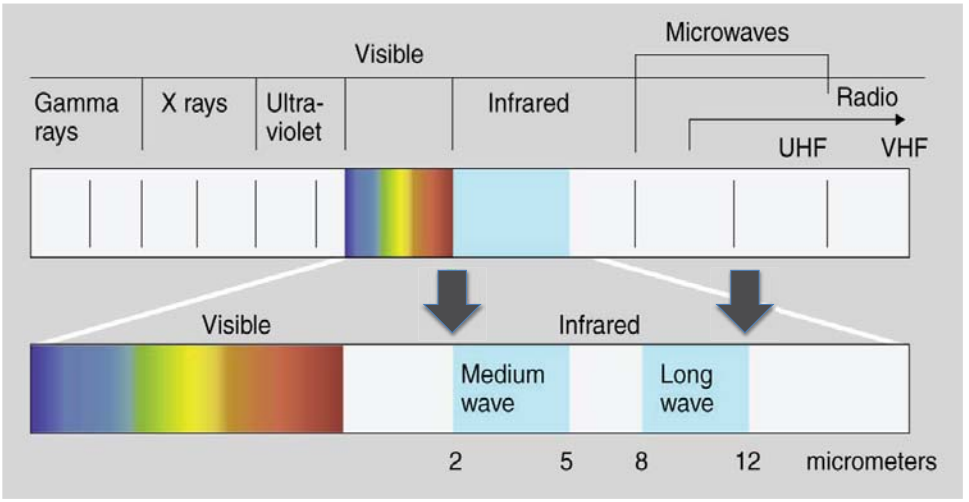

Figure 1 *Electromagnetic spectrum. The beginning and the end of the infrared spectrum indicated with arrows.*

The advantage of thermographic imaging compared to other commonly used diagnostic methods, such as ultrasonography, radiological examination and MRI, is that it is a non-invasive and safe method of detecting and visualising physiological changes that induce superficial temperature changes in animals (Levet et al. 2009). It is a non-contact and non-radioactive method (Saxena & Willital 2008) and does not require sedation of the animal. The method is based on the fact that physiological changes in the animal can lead to changes in blood flow and the following superficial temperature changes can be detected with thermographic imaging (Varjú et al. 2004; Vianna & Carrive 2004; Stewart et al. 2007).

Cell metabolism and local blood flow result in superficial temperature (Van Hoogmoed & Snyder 2002). Superficial skin temperature may change due to many reasons, such as an inflammatory process (Varjú et al. 2004), infection (Arenas et al. 2002; Schaefer et al. 2004; Chiu et al. 2005; Dunbar & MacCarthy 2006; Chiang et al. 2008) or medications (Van Hoogmoed & Snyder 2002; Holmes et al. 2003; Stewart et al. 2009; Figueiredo et al. 2013).

For more than 50 years, thermographic imaging has been used in human medicine to image and visualise pathophysiological changes induced by various diseases and conditions (see review Jiang et al. 2005; see review Ring & Ammer 2012; Simoes et al. 2012), such as breast cancer, diabetic neuropathy, vascular disorders, muscular painful areas, shoulder impingement syndrome, rheumatologic diseases, dry eye syndrome, parasitic and metastatic liver diseases,

bowel ischaemia, renal transplantation and radiation damage to the human body (see review Lahiri et al. 2012). In equine medicine, thermographic imaging has been used for decades (Smith 1964). In small animal medicine, however, the method has not been as widely used. Table 1 presents the essential original articles on animals. Review articles have been published on the observation of zoo animals (Kastberger & Stachl 2003; McCafferty 2007), the imaging of canine stifle (Marino & Loughin 2010), the estimation of metabolic heat loss in birds and mammals (McCafferty et al. 2011), the evaluation of mammalian testes and scrotum (Purohit et al. 2002) and diagnostic thermographics in horses (Turner 2001).

2 Review of the literature

2.1 Background on thermographic imaging

Infrared radiation was defined in the 1800s, which gradually lead to the invention of infrared thermographic imaging (see review Ring 2006). It was originally designed for military purposes (see review Ring 2006), and since the early applications, the development of infrared imaging has advanced considerably (see review Ring & Ammer 2012). The first diagnostic thermographic images were taken by R. Lawson in 1957, with the discovery of an abnormal temperature over a breast tumour (see review Jiang et al. 2005). Medical thermographic images were also taken in 1959–1961 in London in a hospital for rheumatic patients. At the time, taking a thermographic image took 2–5 minutes (see review Ring 2006).

2.2 Principles for thermographic imaging

Thermographic imaging (also known as thermography, infrared thermography and infrared imaging) can be used to measure and visualise superficial temperatures and temperature changes in the body (Stewart et al. 2007).

Changes in superficial temperature or temperature patterns may indicate several illnesses and painful conditions (see review Jiang et al. 2005). For example, an inflammation in subcutaneous and deeper tissues can be reflected as superficial tissue temperature changes of ≥ 1°C (see review Turner 2001; Varjú et al. 2004). The human hand and fingers can detect temperature differences of ≥ 2°C on the skin (Holmes et al. 2003), whereas modern infrared cameras have been claimed to be at least 10 times more sensitive in detecting temperature changes.

Thermographic imaging is a method detecting superficial physiological changes – hence, thermographic imaging is a physiological imaging method. Usually, physiological changes can be noticed before potential anatomical changes will occur. A study by Ringer et al. (2005) indicated that parallel results can be attained by using nuclear scintigraphic imaging, also a physiological imaging method, and thermographic imaging when monitoring the effects of treatment on horse limbs after focused extracorporeal shock wave therapy. When an anatomical change is present, anatomical imaging methods – such as a radiological examination, CT and MRI – are useful in acquiring information.

Table 1 presents studies conducted on dogs, horses, sheep, cattle, llamas, laboratory animals, wild animals, zoo animals and humans.

Table 1 *Original publications on thermographic imaging in different species.*

Species	Object/Condition	References
Dog	Healthy limbs	Loughin & Marino 2007
	Cranial cruciate ligament-deficiency	Infernuso et al. 2010
	Pregnancy	Durrant et al. 2006
Horse	Reliability and repeatability	Tunley & Henson 2004
	Forelimbs, camera angle and distance	Westermann et al. 2013a
	Forelimbs, effect of airflow	Westermann et al. 2013b
	Distal hindlimbs	Ringer et al. 2005
	Inflammatory processes	Purohit & McCoy 1980
	Joint diseases	Vaden et al. 1980; Turner et al. 1983
	Intracynovial injections	Figueiredo et al. 2013
	Injections, neurectomy	Van Hoogmoed & Snyder 2002; Holmes et al. 2003
	Heat loss	Autio et al. 2006, 2007
	Exercise	Simon et al. 2006
	Hoof surface temperature	Douthit et al. 2012, 2014
	Distal limb cast sores	Levet et al. 2009
	Analgesic effect of therapy	Imboden et al. 2009
	Testicles, sperm quality	Ramires Neto 2013
	Pregnancy	Bowers et al. 2009
Cattle	Cow/stress, fear	Stewart et al. 2007, 2008
	Cow/overmilking	Paulrud et al. 2005
	Clinical mastitis	Hovinen et al. 2008
	Calf/pain responses	Stewart et al. 2009
	Calf/infection	Schaefer et al. 2004
	Bull/testicular biopsy	Heath et al. 2001
Sheep	Testicular torsion	Caprano et al. 2008
Llamas	Testicular biopsy	Heath et al. 2002
Laboratory Animals	Mole-rat/surface temperature patterns	Šumbera et al. 2007
	Macaque monkey/emotion	Kuraoka & Nakamura 2011
	Rabbit/stress	Ludwig et al. 2007
	Rat/conditioned fear	Vianna & Carrive 2005
	Sprague-Dawley rat/muscles	Linder-Ganz & Gefen 2007
	Rat/plastic surgery	Tenorio et al. 2009
	Rhesus monkey/nerve injury	Brelsford & Uematsu 1985
	Mouse/tumour	Button et al. 2004; Song et al. 2007
	Rat/intestinal ischaemia	Malafaia et al. 2008
	Pig/burns	Renkielska et al. 2006; Rumiński et al. 2007
Zoo animals	Giant panda/pregnancy	Durrant et al. 2006
	Elephants/ heat loss	Weissenböck et al. 2010
Wild Animals	Hauled-out seals/thermoregulation	Mauck et al. 2003
	13-lined ground squirrel and yellow-bellied marmot/arousal from hibernation	Phillips & Heath 2004
	Fur seals/alopecia syndrome	Lynch et al. 2011
	Raccoons/rabies infection	Dunbar & MacCarthy 2006
	Spanish ibex/sarcoptic mange	Arenas et al. 2002
	Grey seals/instrument attachment	McCafferty et al. 2007
	Grey seals/flipper tag site healing	Paterson et al. 2011

Species	Object/Condition	References
Human	Thermal symmetry	Niu et al. 2001; Vardasca et al. 2012
	Thermal asymmetry	Uematsu et al. 1988a
	Skin temperature	Merla et al. 2002; Gold et al. 2009; Gazerani & Arendt-Nielsen 2011
	Exercise	Ferreira et al. 2008
	Teeth	Kells et al. 2000
	Ocular temperature	Galassi et al. 2007; Chang et al. 2008; Klamann et al. 2012
	Sexual arousal	Kukkonen et al. 2007
	Pregnancy	Simoes et al. 2012
	Paediatrics	Saxena & Willital 2008
	Fever, mass-screening	Chiu et al. 2005; Chiang et al. 2008
	Hot spot identification	Ammer 2011
	Friction blister formation	Hashmi et al. 2013
	Effects of therapy	Hakgüder et al. 2003; Hegedűs et al. 2009; Holey et al. 2011
	Cold intolerance, rewarming	Ruijs et al. 2008
	Cooling of extensive burns	Schnell & Zaspel 2008
	Pain-related phenomena	Devereaux et al. 1986; Pochaczevsky 1987; Uematsu et al. 1988b; Radhakrishna & Burnham 2001; Herry & Frize 2004; Niehof et al. 2006; Park et al. 2007; Meknas et al. 2008; Papež et al. 2009; Wu et al. 2009
	Peripheral nervous system	Uematsu 1985; Galvin et al. 2006
	Nervous-system-related pain	Ammer et al. 2001; Park et al. 2012; Ra et al. 2013
	Malignancy, cancer	Head et al. 2000; Button et al. 2004; Lääperi et al. 2012
	Arthritis	Rusch et al. 2000; Varjú et al. 2004; Spalding et al. 2008; Denoble et al. 2010
	Trauma, compartment syndrome	Katz et al. 2008
	Surgery	Gorbach et al. 2004; de Weerd et al. 2006; 2009; Lee et al. 2007; Iwahashi et al. 2007
	Coronary artery disease	Lawson et al. 1993
	Acupuncture	Yang et al. 2007; Zhang 2007; Agarwal-Kozlowski et al. 2009

2.2.1 Operational principles of infrared cameras

The method of infrared imaging on live animals and humans is based on the phenomenon of infrared radiation from the subject. Radiated heat, or emitted heat (photons), can be detected by an infrared thermographic camera and, consequently, an image can be formed for the naked eye.

2.2.1.1 Emissivity

All animals emit heat through thermal radiation. Heat emitted by an animal (or thermal radiation) may have an internal metabolic source or an external source, such as sunshine or another emitting object. An external heat source is considered to be an artefact in thermographic imaging of animals. Emissivity is the amount of radiation an object emits compared to the radiation of a black body object. The emissivity of a black body object is 1 as it absorbs all radiation and thus appears black in ambient room temperature. The surface of a black body radiator is an ideal limit and does not occur in nature. When an object reflects all radiation, the emissivity is 0. Such an object is also theoretical and does not occur in nature. Accordingly, the emissivity of natural objects remains between 1 and 0.

The emissivity of human skin is between 0.97 and 0.99 (Steketee 1973). The emissivity of animals has not been studied, but an emissivity between 0.95 and 1 has been used in studies (Autio et al. 2006, 2007; Dunbar & MacCarthy 2006; Ludwig et al. 2007; Hovinen et al. 2008; Bowers et al. 2009; Levet et al. 2009; Lynch et al. 2011; Westermann et al. 2013a, 2013b). The value of emissivity can be set on the camera before taking images or with the software when processing images. Emissivity is needed to obtain absolute temperatures from a particular ROI.

2.2.1.2 Formulas substantial for infrared thermographic imaging

In this study, the thermal radiation of animals was measured as emission of heat. Max Planck explained the wave form of thermal radiation in 1900. Thermal imaging as an imaging method is based on this finding. Planck's radiation law can be modelled in terms of black body radiation.

Plank radiation formula

$$S_\lambda = \frac{8\pi\hbar c}{\lambda^5}\frac{1}{e^{\hbar c/\lambda kT}-1} \qquad (1)$$

In the formula:
S_λ = energy per unit, volume per wavelength
π = 3.14159265
c = speed of light (299792458 m/s)
h = Planck constant (6.63 x 10^{-34} Js)
T = temperature in Kelvin
λ = wavelength
k = Boltzmann constant (1.38 x 10^{-23} J/K)

Black body radiation summed over all wavelengths yields the Stefan-Bolzmann's law, which is the very basis of radiation heat transfer and, therefore, the basis of infrared thermographic imaging, since it defines the laws of radiation. Radiated heat follows the formula:

$$P_{rad} = \sigma\varepsilon A_{rad}T^4 \qquad (2)$$

An object can also absorb energy via thermal radiation from the environment:

$$P_{abs} = \sigma\varepsilon A_{abs}T^4 \qquad (3)$$

Taking into account the radiation of the environment (hot object to colder environment), the formula (Stefan-Bolzmann's law) is as follows:

$$P = \sigma \varepsilon A(T^4 - T_{env}^4) \quad (4)$$

In the formulas:
P = Energy emitted (radiated)
σ = Stefan's (or Stefan-Bolzmann's) constant (5.6703×10^{-8} Watt/m^{2}K^4)
ε = emissivity (values between 0 and 1 depending on the composition of the surface)
A_{rad} = radiation area
A_{abs} = absorption area
T = temperature of the radiator
T_{env} = temperature of the environment (surroundings)

2.3 Image capture and processing

2.3.1 Types of thermography cameras

In clinical human and veterinary medicine, the currently used infrared cameras can be divided into two classes: cooled and uncooled matrix detectors.

A cooled infrared camera has detectors commonly manufactured from an alloy of elements, such as mercury-cadmium-telluride or indium-antimonide. The cooling of the detector is currently accomplished with helium in a circulation pump. Older cameras may have required the use of liquid nitrogen for cooling the detector. Sterling engine coolers are also used in thermal cameras. Regardless of the method of cooling, the coolant in the camera detector produces a constant temperature in the detector, providing an absolute temperature reference, and, therefore, cooled cameras can be used to measure the absolute temperatures of the subjects. However, the detectors of cooled cameras need time to cool before imaging and, therefore, the imaging process has to be organised in advance, limiting the use of cooled cameras in clinical practice. Cooled cameras have been used to study squirrels and marmots (Phillips & Heath 2004), pigs (Rumiński et al. 2007), cattle (Schaefer et al. 2004) and humans (Kells et al. 2000; Ammer et al. 2001).

Uncooled thermal camera detectors use pyroelectric or ferroelectric materials, but, typically, microbolometer technology for infrared radiation detection and pixel formation is applied. In uncooled matrix detectors, the function of the detector cell is based on the change of resistance in the detector cell caused by infrared radiation (heat). Uncooled cameras, as opposed to cooled cameras, can be used almost immediately after turning the camera on. Although the characteristics of the cameras are not conclusively reported in several studies on thermographic imaging, uncooled cameras are reported to have been used in medical thermography (Hakgüder et al. 2003; Holmes et al. 2003; Tunley & Henson 2004; Ringer et al. 2005; Simon et al. 2006; Autio et al. 2007; Lee et al. 2007; Loughin & Marino 2007; McCafferty et al. 2007; Stewart et el. 2007; Malafaia et al. 2008; Bowers et al. 2009; Imboden et al. 2009; Levet et al. 2009; Spalding et al. 2009; Stewart et al. 2009; de Weerd et al. 2009; Denoble et al. 2010; Infernuso et al. 2010; Lynch et al. 2011; Douthit et al. 2012, 2014; Figueiredo et al. 2013) due to the lower acquisition price and more flexible usage compared to cooled cameras. The ability of uncooled detectors to calibrate constantly according to the ambient temperature has advanced close to the level of the fixed calibration of cooled cameras, making uncooled cameras suitable for most applications. However, the detectors of uncooled cameras lack the ability to provide a fixed absolute temperature due to

constant calibration (Pedreros et al. 2012). Therefore, measuring the absolute temperature of the subject is not possible without compensatory programmes for the internal temperature of the detector (Pedreros et al. 2012).

The thermal sensitivity, or NETD, of the camera is dependent on the detector properties – the lower the thermal sensitivity, the higher the resolution and accuracy. In cooled cameras, the thermal sensitivity is better than in uncooled cameras. Thermal sensitivity is usually measured and reported as mK.

2.3.1.1 Camera lenses

Infrared radiation does not penetrate ordinary glass. Therefore, special materials have been developed for the lenses of thermographic cameras. The lens optics in basic cameras include, for example, a diamond-like coating or broadband anti-reflective coated germanium, zinc selenidine, zinc sulphide, silicon and calcium fluoride. In addition, plastic lenses are being developed. The lens material enables the lens to transmit and refract radiated heat.

2.3.1.2 Spectral range

Infrared radiation can be divided into near-infrared radiation (0.75–1.4 μm), short-wavelength infrared radiation (1.4–3 μm), mid-wavelength infrared radiation (3–8 μm) and long-wavelength infrared radiation (8–15 μm). Uncooled thermal cameras with a microbolometer detector usually operate in the long-wavelength range, with a few exceptions. With some cooled cameras, it is possible to measure mid-wavelength infrared radiation, depending on the camera model.

2.3.1.3 Spatial resolution

Spatial resolution, or IFOV, or Spot Size determines the smallest target that a camera can detect at any given distance. The spatial resolution can be calculated as the lens angulation divided by the horizontal resolution. The value of spatial resolution is given in mRad. For example, if the spot size ratio is 1000:1 mm, the minimal visible object from a distance of 1 m is 1 mm. The resolution power is inversely proportional to the known width of the object and to the horizontal resolution of the camera. Accordingly, the same data from a ROI using different distances from the object can be gained from the image as long as the ROI covers the same amount of pixels at every distance used. In other words, in order to get similar data on the object from different distances, a camera lens with a smaller angle of view should be used when imaging an object from a greater distance and a camera lens with a wider angle of view for objects closer to the camera.

2.3.1.4 Resolution

If an infrared camera is reported to have a resolution of 320 x 240 pixels, the camera's image sensing device (detector) consist of an array of 320 x 240 light-sensing detectors at the focal plane of the lens (FPA). The resolution of the FPA contributes to the limitations of spatial resolution. Due to this limitation, the temperature of small targets may be difficult to determine.

The camera resolution used for studying small-sized animals has often been 320 x 240 pixels (Loughin & Marino 2007; Malafaia et al. 2008; Infernuso et al. 2010). Resolutions for equine or cattle studies has been reported to be from 80 x 60 (Levet et al. 2009) and 320 x 240 pixels (Autio et al. 2007; Stewart et el. 2007, 2009; Imboden et al. 2009; Ringer et al. 2009) to 640 x 480 pixels (Douthit et al. 2012, 2014).

2.3.2 Camera lens angle and distance of the subject

Since a rounded surface of the object has an effect on the images (Watmough et al. 1970), the camera angle should be kept constant to obtain comparable images. A change in the angle in comparison to the ROI will affect the amount of pixels gained from that area. In many studies, the perpendicular angle (90°) has been used (Holmes et al. 2003; Gorbach et el. 2004; Varjú et al. 2004; Simon et al. 2006; Stewart et el. 2007). However, an angle other than 90° has been used to mimic the positioning for a radiological examination of the ROI (Denoble et al. 2010). In addition, a minor change (20°) in camera angle has been reported to have no significant effect on the temperatures obtained from the images of horses (Westermann et al. 2013b).

Distance also has an effect on the obtained thermographic images. Depending on the camera and software used, distance usually merely affects the amount of pixels received from the ROI. This, however, might influence the interpretation. Various distances used in human studies are presented in Table 2.

Table 2 *Various distances used for thermographic imaging in different species.*

Distance	ROI	Species	References
0.9 m	Face	Human	Zhang 2007
0.05	Teeth	Human	Kells et al. 2000
0.383 m	Hand	Human	Varjú et al. 2004
0.20 m	Eye	Human	Klamann et al. 2012
1-2.34 m	General view	Human	Hakgüder et al. 2003 Ferreira et al. 2008 Dibai Filho et al. 2013
0.279 m	Testicles	Sheep	Caprano et al. 2008
0.6–0.9 m	Abdomen	Giant panda Dog	Durrant et al. 2006
0.5–1 m	Eye	Cow	Stewart et el. 2007
0.7 m	General view, above	Rat	Vianna & Carrive 2005
1.5–4.6 m	Depending on ROI	Dog	Loughin & Marino 2007
0.6 m	Flipper	Grey seal	Paterson et al. 2011
1–3 m	General view	Grey seal	McCafferty et al. 2007
1–2 m	Limbs	Horse	Holmes et al. 2003 Ringer et al. 2005 Imboden et al. 2009 Levet et al. 2009 Figueiredo et al. 2013 Westermann et al. 2013a
8 m	General view	Horse	Autio et al. 2007

More specific areas are imaged from a smaller distance than the more general views. A study conducted on horses suggests that an increase of 1–1.5 metres in distance has no significant effect on the temperature obtained from the images (Westermann et al. 2013a).

2.3.3 Emissivity in medical thermographic imaging

The emissivity of human skin of any ethnicity has been investigated and is reported to be almost constant with a value of 0.98±0.01 for wavelengths of 2–14 μm (Steketee 1973). Inflamed skin, such as burned skin, has the same emissivity as healthy skin (Steketee 1973). The emissivity of 0.98 is therefore used in imaging humans (Galvin et al. 2006; Dibai Filho et al. 2013). Human enamel has been reported to have the calculated emissivity of 0.65 (Kells et al. 2000). The exact effect of the application of cosmetics to human skin has not been specified and, therefore, human subjects are asked to refrain from using cosmetics before thermographic imaging (Watmough et al. 1970; Ferreira et al. 2008; Gazerani & Arent-Nielsen 2011; Vardasca et al. 2012).

In animals, the emissivity has not been thoroughly defined. Supposedly for this reason, the applied emissivity has not been reported in all published studies. Estimated emissivity values have been used in animal studies. In horses, the values of 0.95 (Autio et al. 2006, 2007), 0.96 (Bowers et al. 2009), 0.98 (Westermann et al. 2013a) and 1 (Levet et al. 2009; Westermann et al. 2013b) have been applied. In seals (Lynch et al. 2011; McCafferty et al. 2007), cows (Hovinen et al. 2008) and raccoons (Dunbar & MacCarthy 2006), the emissivity of 0.98 has been used, while a value of 0.97 has been applied to rabbits (Ludwig et al. 2007).

2.3.4 Temperature regulation of animal body

2.3.4.1 Basic physiology of temperature balance

Basic physiological functions of the animal body regulate the heat balance. Some bodily functions, such as muscle work, generate excess heat that needs to be eliminated in order to maintain the homeostasis and normal functions of the body. Different methods exist for removing the heat generated in the body.

A large proportion of the metabolic energy of animals and humans is transferred from the body to the surroundings as heat by means of radiation, convection, conduction and evaporation (sweating, panting) (Hammel 1955, 1958; Taylor et al. 1971). Vasodilatation of the peripheral vascular system assists in the release of heat from the body (Hammel et al. 1958).

Thermographic imaging has been used to demonstrate that, in birds and mammals, heat loss is regulated from poorly insulated regions of the body. This heat loss can be observed as warmer areas, thermal windows, in thermographic images of the animal (Weissenböck et al. 2012; see review McCafferty et al. 2011).

Cell metabolism and local blood flow result in the temperature of an area (Van Hoogmoed & Snyder 2002). The superficial blood flow can be enhanced for any physiological or pathophysiological reason resulting in increased skin temperature (Varjú et al. 2004; Vianna & Carrive 2005). Superficial skin temperature rises in the inflammatory process due to vasodilatation of blood vessels (Varjú et al. 2004) and the increased rate of blood flow (Vianna & Carrive 2005). The enhancement in peripheral blood flow leads to heat loss, which can be detected with thermographic imaging (Autio et al. 2006; Stewart et al. 2007).

2.3.4.2 Significance of the hair coat to heat loss

A thick hair coat protects the trunk of the animal from excessive heat loss (Henshaw et al. 1972). Insulating fur may even prevent heat loss and force animals to find alternative ways of cooling down. Hair coat weight has been demonstrated to correlate negatively with heat loss (Autio et al. 2007). In horses, differences in heat loss between horse breeds at low temperatures as well as the

rate of heat loss during seasons have been suggested (Autio et al. 2006). The length of hair also affects the time it takes the horse to acclimatize to the environment (Tunley & Henson 2004).

Good insulation with the aid of fur may prevent the detection of superficial vasomotor changes in an animal with thermographic imaging, as suggested by Phillips & Heath (2004) studying temperature changes during arousal from hibernation in two different-sized species, the 13-lined ground squirrel (225±12 g) and the yellow-bellied marmot (2.26±1.05 g).

2.3.4.3 Foot pad thermoregulation

In the arctic fox and gray wolf, thermoregulation through the foot pad appears to be regulated with a circulation mechanism separate from the deeper metatarsal arteries (Henshaw et al. 1972). The surface temperature of a foot pad is regulated by means of vasodilatation and differential pumping of heat from the circulating blood even when there is an added energy cost to the animal (Henshaw et al. 1972). It is not known whether the same mechanisms appear in domestic canines.

2.3.5 Preconditions for the subject before thermographic imaging

Thermographic images are sensitive to artefacts. Therefore, humans have been instructed to refrain from certain behaviours that may affect blood flow hours before the thermographic imaging, such as drinking alcohol, smoking (Gold et al. 2009; Gazerani & Arent-Nielsen 2011; Vardasca et al. 2012), consuming caffeine or other hot or cold drinks (Gazerani & Arent-Nielsen 2011), eating heavy meals, performing heavy exercise or physiotherapy (Vardasca et al. 2012), applying any lotions (Ferreira et al. 2008; Gazerani & Arent-Nielsen 2011) or similar products such as oil ointments or cosmetics on the ROI, or wearing jewellery on the examination day (Vardasca et al. 2012) to avoid artefacts.

The effects of exercise on thermographic images are evident (Vaden et al. 1980) but, usually, they wane off within a certain time. This was discovered from images of horses doing high-speed treadmill exercise: within less than 45 minutes, the baseline values for the proximal and distal limbs were restored (Simon et al. 2006). Accordingly, to prevent artefacts, it is suggested that field-kept horses be taken inside, any rugs or bandages removed, mud and debris removed from the coat and the hair coat thoroughly dried from rain or sweat, and that the horses be kept away from sunlight for at least an hour before thermographic imaging (Tunley & Henson 2004). It would be ideal for the animals to be imaged in a draught-free area with low energy emitting lighting and a 20°C ambient temperature (Ringer et al. 2005). Furthermore, a feeding restriction for 2 hours before imaging has been applied to horses to avoid postprandial variation in thermal patterns (Holmes et al. 2003).

2.3.5.1 Acclimatization of the subject

Acclimatization of the subject to the surrounding environment is essential in order to produce standardized, good-quality images. Different acclimatization times have been used depending on the species, the ROI and the place where the imaging takes place. In studies conducted on humans, the frequently-used acclimatization times have been 10 minutes (Ferreira et al. 2008; de Weerd et al. 2009; Denoble et al. 2010), 15 minutes (Kells et al. 2000; Hakgüder et al. 2003; Varjú et al. 2004; Lee et al. 2007; Park et al. 2007; Holey et al. 2011; Park et al. 2012; Simoes et al. 2012; Ra et al. 2013), 20 minutes (Devereaux et al. 1986; Niu et al. 2001; Gold et al. 2009) and 30 minutes (Agarwal-Kozlowski et al. 2009). In order to study eye temperature in humans, subjects

were asked to close their eyes for 3–5 seconds (Galassi et al. 2009; Klamann et al. 2012). A similar procedure is not practical with animals, since animals cannot be instructed to close their eyes.

The acclimatization times for horses have been reported to be less or equal to 30 minutes (Van Hoogmoed & Snyder 2002; Ringer et al. 2005; Imboden et al. 2009; Figueiredo et al. 2013; Westermann 2013a, 2013b) or between 39 and 60 minutes (Tunley & Henson 2004), although longer periods, even hours, have also been used (Holmes et al. 2003; Autio et al. 2006; Douthit et al. 2014). The acclimatization time is dependent on the temperature difference between the environment and the subject – the smaller the temperature difference between horse's environment and the thermographic examination area, the shorter the time it takes for the animal to become acclimatized (Tunley & Henson 2004). However, in horses, the time used for acclimatization did not affect the thermal patterns recorded (Tunley & Henson 2004). For bulls and mole rats, an acclimatization period of 30 minutes before thermographic imaging has been used (Heath et al. 2001; Šumbera et al. 2007).

2.3.5.2 Cold challenge as a tool to monitor changes in blood flow

In order to acquire information on blood flow and perfusion, the ROI can be challenged to cold and the rewarming period monitored with infrared imaging (Rusch et al. 2000; de Weerd et al. 2009). In inflamed human joints in rheumatoid arthritis, two different rewarming processes have been detected – the faster representing the rewarming due to arterial blood flow and the slower the additional rewarming by pathological venous skin blood flow due to excessive nitric oxide production in inflamed tissues (Rusch et al. 2000). Promising results have been obtained with the use of cold provocation in a study on human breast cancer: it takes longer for the cancerous tissue to return to baseline temperature after cold provocation than normal healthy tissue (Lääperi et al. 2012).

2.3.5.3 Hair clipping

Although clipping the hair from the ROI has been found to affect the mean temperature of the area, thermal patterns of the ROI remained similar before and after the hair clipping in dogs (Loughin & Marino 2007) and in horses (Turner et al. 1983). This information has been applied for pregnancy detection when the hair has been clipped from the abdominal area to image possible pregnancy of giant pandas and domestic canines without hair clipping (Durrant et al. 2006).

2.3.6 Image processing and interpretation

Software for the processing of thermographic images enables temperature measurement using different shapes over the ROI (point, rectangle, oval). From these shapes (rectangle, oval), the software calculates mean values, the highest and lowest temperatures that can be used for evaluating the image (Holmes et al. 2003; Westermann et al. 2013a). Mean temperature values for ROIs (Ammer et al. 2001) or, for example, homogenous areas of 20–30 pixels can be used for temperature measurement and comparison of different temperatures obtained from the ROIs (Ludwig et al. 2007).

The development of new software and techniques for image analysis in medical thermographic imaging is an ongoing process. Image processing has an impact on the

reproducibility of certain phenomena, such as hot spots (Ammer 2011). A poor contrast between the target and its background will make it difficult to observe the ROI and increase the time used in image analysis (Bajwa et al. 2010).

However, basic image processing software delivered with the thermal camera or downloaded from the internet has been successfully utilised for animals and humans (Ringer et al. 2005; Hashmi et al. 2012; Dibai Filho et al. 2013; Figueiredo et al. 2013). In some studies, more advanced purchasable software (Autio et al. 2007; Stewart et el. 2007; Song et al. 2007; Gold et al. 2009; de Weerd et al. 2009; Levet et al. 2009; Infernuso et al. 2010; Gazerani & Arendt-Nielsen 2011; Simoes et al. 2012; Westermann et al. 2013a) or software developed for the study has been used (Ferreira et al. 2008; Vardasca et al. 2012). Some computerised assessment techniques for assessing pain-related thermal dysfunction based on thermographic images (Herry & Frize 2004) or other illnesses (Infernuso 2010) have already been developed.

2.3.6.1 Typical artefacts

Natural or artificial phenomena can induce artefacts in thermographic images and should be taken into consideration when taking and interpreting the images. The environment where the thermographic imaging takes place should be controlled in respect to examination room temperature, lighting and draught (see review Lahiri et al. 2012), since even subtle airflow can have an effect on thermographic images by decreasing the temperature of the ROI in relation to the air velocity (Westermann et al. 2013a).

Frost on the surface of an animal's hair coat may affect the thermal images (Autio et al. 2007), especially when it thaws resulting in a moist surface that enhances evaporation form the surface. However, it is possible to obtain thermographic images of a marine mammal when the imaged part is above the surface of the water (McCafferty et al. 2007; Lynch et al. 2011).

Infrared energy from the environment can be minimised by using fluorescent lighting to minimise the emitted thermal energy from the light source and, for example, black velvet wall-covering behind the subject to prevent reflections onto the subject from the surrounding surfaces (Denoble et al. 2010). The application of an external heat source enhances local perfusion by means of vasomotor responses in human skin, creating an artefact which can be detected by thermographic imaging (Gazerani & Arendt-Nielsen 2011).

Standard indoor illumination and no draught have often been considered adequate (Klamann et al. 2012). When considering the Stefan-Bolzmann's law, basically any external factor that influences the emitted heat of the subject or the heat balance between the surroundings and the subject can cause artefacts to the images.

Intentionally created artefacts can be used to reliably identify the ROI. An anatomical marker (a 2 cm diameter silver sticker) has been employed to obtain an image for reference in studying human knee osteoarthritis (Denoble et al. 2010).

2.3.6.2 Temperature symmetry and asymmetry

In a healthy human body, there is contralateral symmetry in skin temperature (Herry & Frize 2004) and ocular temperature between the right and left eye (Klamann et al. 2012). When comparing the selected skin regions between the sides, thermal asymmetry has been found to be very small in normal, healthy humans (Uematsu et al. 1988a). Depending on the ROI, the normal thermal asymmetry in humans has been found to be from 0.17±0.14°C (abdomen) to 0.67±0.55°C (fifth toe) (Uematsu et al. 1988a). This data was derived using different kinds of

thermal cameras, and the temperature results were discovered to remain similar (Uematsu et al. 1988a) despite the variation in equipment. In other studies, a difference exceeding 0.3°C (Lee et al. 2007) or 0.5°C (Hakgüder et al. 2003) between the right and left side has been considered abnormal in humans. A recent study on healthy human male subjects indicated that a temperature symmetry difference was 0.4°C±0.3°C at the most in whole body images and 0.4°C±0.15°C at the most in regional images (Vasdasca et al. 2012).

The maximum thermal symmetry difference has been defined to be 0.3±0.2°C in healthy pregnant women, which coincides with non-pregnant women (Simoes et al. 2012). However, the thermal pattern appears to be different in pregnant women, especially in the anterior view, due to changes in the body in the late gestation period (Simoes et al. 2012). Although age has not been discovered to influence the temperature results obtained from thermographic images, body mass index as well as foetal weight and positioning may have an effect on the distribution of body heat (Simoes et al. 2012).

Differences greater than those found in healthy humans might be an indicator of a deviant condition, such as a disease or a painful process. Most neuropathic pain conditions and some nociceptive pathologies can be associated with changes in the thermal distribution of the body (Herry & Frize 2004). For example, in patients with peripheral nerve injury, the temperature of the skin innervated by the damaged nerve can vary by an average of 1.55 °C, being colder or warmer depending on the type of nerve damage (Uematsu 1985). A significant temperature difference between the affected and unaffected side in a human patient with herpes zoster or post-zoster neuralgia can be as small as 0.52±0.30°C (Ammer et al. 2001).

Thermographic symmetry has been evaluated in the limbs of healthy dogs, and no significant difference between the left and right legs was found, suggesting that thermal abnormalities between the sides could be used as a diagnostic tool for dogs (Loughin & Marino 2007). For healthy ridden and unridden horses, topographical thermographic patterns and reference values have already been obtained (Tunley & Henson 2004).

2.4 Effects of some physiological and pathophysiological phenomena on thermographic images in humans and animals

2.4.1 Inflammation

Inflammation can be seen as an increased superficial temperature in thermographic images (Varjú et al. 2004; Vianna & Carrive 2005). In a previous study by Purohit and McCoy (1980) thermographic imaging was found to be effective in quantitative and qualitative evaluation of inflammatory processes in horses. In the inflammatory state of Grave's ophtalmopathy, human patients were treated with immunosuppressive methylprednisolone pulse therapy, which decreased the temperature of the eye and its surrounding tissues as an effect to treatment (Chang et al. 2008).

2.4.2 Infection

Signs of clinical *Escherichia coli* lipopolysaccharide-induced mastitis can be detected with thermographic imaging, but local signs, such as pronounced swelling, have been detected without a camera before the elevation of udder skin temperature or body temperature (Hovinen

et al. 2008). However, thermographic imaging might be useful in detecting clinical mastitis or other diseases with an increased superficial temperature in dairy cows (Hovinen et al. 2008).

In humans, thermographic imaging was an effective and reliable tool for mass-screening patients with fever in the initial phase of severe acute respiratory syndrome (Chiu et al. 2009). However, when the screening suspected febrile patients, thermographic imaging may have yielded false negative results and decreased the sensitivity for screening fever if the febrile patient was sweating, but, in spite of this limitation, thermographic imaging can be used as a proxy for core temperature in humans (Chiang et al. 2008).

The method of thermographic imaging has been discovered to be useful in remote detection of infectious diseases in animals (Schaefer et al. 2004; Dunbar & MacCarthy 2006). A temperature increase in the nose of raccoons experimentally infected with rabies was evident concurrently with clinical signs of rabies (Dunbar & MacCarthy 2006). A significant increase in facial area temperature has been observed in consequence to Type 2 bovine viral diarrhoea virus in calves (Schaefer et al. 2004). However, in diagnosing sarcoptic mange in wild Spanish ibexes, thermographic imaging was not found to be an as sensitive a method as conventional binoculars due to the great distance from the observer to the animal (Arenas et al. 2002).

2.4.3 Wound healing

Wound healing has been monitored by means of thermography. Increased temperature in a healing wound without infection was observed in grey seals (Paterson et al. 2011). Furthermore, any signs of possible infection can be monitored with thermographic imaging as has been done with grey seals after flipper tag placement (Paterson et al. 2011).

2.4.2 Effects of physical exercise

The effects of localised exercise, such as knee extension and flexion, in young and elderly humans have been studied using thermographic imaging (Ferreira et al. 2008). The results indicated that, even though younger subjects had a higher pre-exercise temperature, both young and elderly subjects had a similar capacity to produce heat, which could help us to understand the temperature changes in elderly subjects and also aid in sports and rehabilitation programmes (Ferreira et al. 2008).

2.4.3 Musculoskeletal disorders

2.4.3.1 Disorders of the muscles

Injuries to the muscles may induce changes in blood flow and, therefore, thermographic imaging can be employed to detect muscle injuries (see review Steiss 2002). In animals with a small body size, such as mice, deep tissue injuries induced by pressure or shear of the tissue of skeletal muscles, have also be detected (Linder-Ganz & Gefen 2007). Thermographic imaging has been discovered to be reliable in a controlled ambient environment when assessing the severity of upper extremity musculoskeletal disorder in office workers (Gold et al. 2009). However, studying the masticatory muscles of humans, thermographic imaging proved not to be an accurate instrument for myogenous temporomandibular disorder (Dibai Filho et al. 2013) or to correlate with pressure sensitivity in myofascial tenderness (Radhakrishna & Burnham 2001).

2.4.3.2 Disorders of the joints

Thermographic imaging has been found to provide reliable measurements of the thermal patterns of arthritic joints in humans (Spalding et al. 2008). The thermographically measured surface temperatures of osteoarthritic hand joints in humans were higher at the onset of osteoarthritis due to the initial inflammatory process and declined as the severity of radiological osteoarthritis worsened after the initial phase of the disease (Varjú et al. 2004). In human osteoarthritic knee joints, the skin's surface temperature in the affected knee correlated significantly with the severity of the defects of the knee observed in a radiological examination (Denoble et al. 2010).

Thermographic imaging has been found reliable and objective in measuring the sings of inflammation in the knee joint in humans (Denoble et al. 2010), and it has also been used for diagnosing cranial cruciate ligament rupture in dogs with no clinical findings of stifle laxity (Infernuso et al. 2010). It can also be used to monitor the pathological processes in joints and to evaluate the efficacy of anti-inflammatory medication in human patients with rheumatoid arthritis (Rusch et al. 2000).

Thermographic imaging can be employed to objectively reflect the pathological processes of shoulder stiffness in patients with shoulder impingement syndrome (Park et al. 2007) and has been found to provide a reliable and objective method for diagnosing elbow tendinosis and evaluating the outcome after treatment (Meknas et al. 2008). Furthermore, thermographic imaging has been demonstrated to be a sensitive and reproducible method for confirming the clinical diagnosis of patellofemoral arthralgia in its early stages, thus eliminating the need for invasive methods (Devereaux et al. 1986).

2.4.4 Cardiovascular changes in the body

2.4.4.1 Conditions affecting circulation

Circulatory changes in the skin may result from trauma that has disturbed the sympathetic nervous system controlling the microcirculation of the skin. Early diagnosis can be made using thermographic imaging before skin or radiologically detectible changes have developed (Pochaczevsky 1987). This enables early and effective treatment before more serious disabilities develop (Pochaczevsky 1987). Thermographic imaging can also aid in the differentiation of false positive human patients from real positive ones with reflex sympathetic dystrophy when both the radiological and other examinations produce no findings (Pochaczevsky 1987).

In horses, thermographic imaging can detect changes in blood flow in the hoof, as ultrasonographic measurements of the velocity of digital blood vessels correlate with hoof temperature measured with thermographic imaging (Douthit et al. 2014).

In humans, thermographic imaging has been applied when monitoring changes in microcirculation in the manipulated area after therapeutic treatment, such as low-level laser therapy (Hakgüder et al. 2003; Hegedüs et al. 2009) and connective tissue massage (Holey et al. 2011). Vasoconstriction of small arteries and arterioles in the extremities can result in visible colour changes due to circulatory changes in the affected skin area, which can be monitored with thermographic imaging (Merla et al. 2002). Thermographic imaging has also been considered to be useful in investigating the link between coronary ischaemia, nervous system activation and perception (Lawson et al. 1993). It may also prove to be useful for the evaluation of the pulpal status, since it has been established to be suitable for imaging vital teeth (Kells et al. 2000).

2.4.4.2 Psychological stress

Stress-induced vasoconstriction has been found to cause a detectable decrease in the ear temperature of rabbits after a stressful event (Ludwig et al. 2007) and in the nasal temperature of macaques during negative emotional states (Kuraoka & Nakamura 2011). This study by Kuraoka and Nakamura (2011) suggests that a decrease in nasal skin temperature should be considered as an indicator of emotional states in animals. Skin temperature has also been shown to decrease as a reaction to fear in rats through cutaneous vasoconstriction in the tail and paws (Vianna & Carrive 2005).

An eye temperature increase in cows was recorded with thermographic imaging after a stressful situation, such as jugular vein catheterization, as opposed to an external medically induced stimulus such as corticotrophin-releasing hormone and adrenaline (Stewart et el. 2007). These results indicate that, in addition to the activation of the hypothalamic-pituitary-adrenal axis, a cognitive component may also be required in bovines to produce an eye temperature response to stress (Stewart et el. 2007). However, an eye temperature decrease due to a sympathetic response in cows has been observed after stimulating fear and pain (Stewart et al. 2008). These results suggest that thermographic imaging could be used to assess fear and pain responses to handling in cattle (Stewart et al. 2008).

2.4.4.3 Physiological stress

Pregnancy causes changes in the body and circulation, which can be observed with thermographic imaging (Durrant et al. 2006; Bowers et al. 2009). Pseudopregnancy and pregnancy in giant pandas has been successfully detected and distinguished (Durrant et al. 2006), but with domestic canines, thermographic images were not conclusive in detecting pregnancy (Durrant et al. 2006). In pregnant horses, the flank area temperature has been demonstrated to be significantly higher and the thermal pattern more irregular in comparison to non-pregnant mares (Bowers et al. 2009).

Galassi et al. (2009) detected that ocular surface temperature was significantly lower in human patients with primary open-angle glaucoma than in controls and suggested that a decreased ocular surface temperature could be used as a marker for impaired retrobulbar haemodynamics in glaucoma patients.

2.4.5 Trauma and surgery

2.4.5.1 Trauma

According to a study on friction blister formation in humans, it has been suggested that thermographic imaging could be a useful tool in the remote assessment of the inflammatory responses of traumatically damaged foot skin (Hashmi et al. 2013). Furthermore, thermographic imaging has been established to be a valuable and rapid clinical tool for monitoring the development of cast sores in the distal limbs of horses (Levet et al. 2009).

Trauma with swelling of muscles within confined compartments, such as in the legs, can lead to elevated pressure in the compressed area, causing ischaemia which leads to compartment syndrome (Katz et al. 2008). The resulting skin surface temperature changes in humans can be detected with thermographic imaging (Katz et al. 2008). Early detection of compartment syndrome is essential in order to avoid amputation or death and, therefore, thermographic

imaging could be used as a supportive tool for early detection of acute compartment syndrome in trauma patients (Katz et al. 2008).

With the aid of a pig skin model, thermographic imaging has been established to be able to distinguish the shallower burns that would heal spontaneously within 3 weeks and deeper wounds that would require further treatment (Rumiński et al. 2007). This method could be applied in clinical human practice to burn trauma patients (Renkielska et al. 2006). In humans, tissue damage induced by burning is initially treated with sprayed or poured coolants (Schnell & Zaspel 2008). With the use of thermographic imaging, sprayed coolants have been found to be significantly more efficient in decreasing the skin temperature than poured coolants in healthy test subjects (Schnell & Zaspel 2008).

2.4.5.2 Surgery

Thermographic imaging has been utilised intraoperatively to monitor blood flow, such as cerebral blood flow in the cortex during brain tumour surgery (Gorbach et al. 2004) and the restoration of blood supply to the heart in coronary bypass surgery (Iwahashi et al. 2007) in humans. Furthermore, intraoperative monitoring with thermographic imaging during plastic surgery (free flap surgery) provided the surgeon with valuable information on the reperfusion of the flap (de Weerd et al. 2006). A study conducted on rats suggested that thermographic imaging can be used postoperatively for the early detection of free flap failure and to aid in the salvage of the flap, since early recognition of perfusion failure is essential for flap salvage (Tenorio et al. 2009). Thermographic imaging can also be applied to monitor skin perfusion after other surgical procedures, such as autologous breast reconstruction (de Weerd et al. 2009).

The effects of surgical biopsies have been evaluated using thermographic imaging in bulls (Heath et al. 2001), llamas (Heath et al. 2002) and grey seals (McCafferty et al. 2007). After testicular biopsy in bulls and llamas, no significant differences in the normal heat pattern of the testes between the biopsied and the non-biopsied testes could be detected with the aid of thermographic imaging (Heath et al. 2002). Normal heat patterns of functional testes have been presented for bulls and stallions (Ramires Neto et al. 2013), as well as bucks, dogs and llamas (see review Purohit et al. 2002). With the knowledge of the normal temperature pattern of testes, the significant testicular cooling in testicular torsion has been detected and diagnosed in sheep using thermographic imaging (Caprano et al. 2008). In grey seals, the biopsy site was visible with thermographic imaging after blubber biopsy, but no long-term changes in temperature were observed (McCafferty et al. 2007).

2.4.5.3 Paediatric patients

Thermographic imaging has proved to be an excellent tool in the follow-up of, for example, haemangiomas, vascular malformations and digit amputations related to reimplantation in paediatric human patients (Saxena & Willital 2008). In emergency situations, it has been found to be valuable in the rapid diagnosis of extremity thrombosis, varicoceles, inflammation, abscesses, gangrene and wound infections (Saxena & Willital 2008). As a non-contact method, thermographic imaging has been well tolerated by frightened children for the temperature evaluation of a ROI, and the colour images have also enabled the explanation of the disease to older children and their parents without complicated medical terminology (Saxena & Willital 2008).

2.4.6 Tumours

Initial studies conducted on mice and humans have indicated that thermographic imaging could be useful in cancer detection, especially if the cancerous process involves a release of nitric oxide in dilating blood vessels (Button et al. 2004) and enhancing heat emission. If the tumour has an impaired blood supply due to smaller size, poor organisation and hyperpermeability of the blood vessels, it can appear colder than the surrounding tissues in the thermographic images, as suggested with Song et al. (2007).

Although not accepted as the only diagnostic modality in breast cancer detection, thermographic imaging can be used for risk assessment – women with abnormal images are at a greater risk (close to 30%) of developing breast cancer (Head et al. 2000). Using an advanced integrated technique, thermographic imaging has been found to have an accuracy of 80.95%, with 100% sensitivity and 70% specificity, in identifying breast cancer, whereas a clinical examination conducted by an experienced radiologist has an accuracy rate of 60%–70% (Ng & Kee 2008).

2.5.7 Neurological dysfunctions

In humans, nerve impairment can be classified into three categories according to the type of nerve injury: complete interruption of all nerve fibres including sympathetic fibres when the injured area has an increased temperature surrounded by a colder area; partially impaired sympathetic function when the injured area appears diffusely warmer with colder surroundings; and an interruption of the neighbouring nerves with normal sympathetic function in the area of intact nerves when thermographic images show a colder area in the centre surrounded by increasingly warmer zone (Uematsu 1985). Thermographic imaging can help in assessing thermoregulation after injury to peripheral nerves, with images taken after cold exposure followed by monitoring the rewarming process, indicating that an area innervated by injured nerves heats up more slowly than the uninjured side (Ruijs et al. 2008).

Thermographic imaging has been used to study and document cutaneous projections of sensory and sympathetic dysfunction in peripheral nerves in primates (monkeys) (Brelsford & Uematsu 1985). Although thermographic imaging cannot be recommended as a diagnostic tool for the severity of carpal tunnel syndrome in humans, it could be used as a screening tool (Papež et al. 2009). Furthermore, it can be used to visualize human lower back problems, such as pain and sciatica (Uematsu et al. 1988b).

Thermographic imaging has been suggested as a potential complementary tool in conjunction with clinical and MRI findings for the diagnosis of unilateral lumbosacral radiculopathy in humans (Ra et al. 2013) and in evaluating the injury and recovery of orthognathic patients with an inferior alveolar nerve injury (Lee et al. 2007). It can also be employed to visualize and study meridian-like structures and acupoints (Yang et al. 2007), to select acupoints (Zhang 2007) and to distinguish the needling of true acupoint (temperature increase after needling) and nonacupoint (temperature decrease after needling) (Agarwal-Kozlowski et al. 2009).

Thermal asymmetry of the skin can be observed in patients with acute herpes zoster and post-zoster neuralgia (Ammer et al. 2001). In most human patients, the thermal patterns in acute herpes zoster appear warmer than the control side, but they can also be colder (Ammer et al. 2001). In human shoulder impingement syndrome, thermographic images can show hypothermia or hyperthermia in the affected area, the hypothermic patients having more limited shoulder movement (Park et al. 2012).

When evaluating the success and failure of axillary regional blockades using mepivacaine in humans, thermographic imaging has proved to be more sensitive and specific, in addition to having higher positive and negative predictive values, when compared to pinprick and cold sensation evaluations (Galvin et al. 2006).

In brief, thermal imaging has been successfully used as an aid in diagnosing and, in certain cases, also distinguishing between different types of nerve-related phenomena (Uematsu 1985; Ammer et al. 2001; Ruijs et al. 2008; Agarwal-Kozlowski et al. 2009; Park et al. 2012).

3 Aims of the study

The main objective of this thesis was to investigate the usability and clinical applications of thermographic imaging for small animals in clinical veterinary medicine. More detailed aims were as follows:

1. To compare three thermal cameras with different resolutions and to determine which resolution would be best-suited for veterinary purposes. (I)

2. To investigate the amount of variance caused by thermographers and interpreters of thermographic images and to evaluate the impact of that variance on the clinical use of thermographic imaging. (I)

3. To establish the usability of the freeware QuickReport 2.1 in the interpretation of thermographic images obtained from dogs. (I)

4. To show that the variance caused by animals (imaging subjects) would be small enough to be considered negligible in clinical use of thermographic imaging (I) and, further, to investigate the possibilities of using thermographic imaging and thermal images clinically in cats (III) and dogs (II, IV).

4 Materials and methods

4.1 Equipment and thermographers

4.1.1 Thermal cameras

Three types of thermal cameras were preliminarily tested. The cameras used were FLIR i5, FLIR b60 and FLIR T425 (FLIR Systems Inc., Sweden). The specifications of the thermal cameras are shown in Table 3. Based on the preliminary results, the camera with the highest resolution was used in the subsequent studies.

Table 3 *Specifications of the thermal cameras used in study I.*

	i5	**b60**	**T425**
IR resolution	80 x 80 pixels	180 x180 pixels	320 x 240 pixels
Thermal sensitivity/NETD	<0.1°C/100 mK	<0.07°C/70 mK	<0.05°C/50 mK
Spatial resolution (IFOV)	3.7 mRad	2.42 mRad	1.36 mRad
Accuracy	±2°C or ±2% of reading	±2°C or ±2% of reading	±2°C or ±2% of reading
Detector type	Uncooled microbolometer	Uncooled microbolometer	Uncooled microbolometer
Image frequency	9 Hz	9 Hz	9 Hz

4.1.2 Thermometers

A digital rectal thermometer for animals (Vet-Temp 1831, Microlife, Widnau, Switzerland) was used to measure the rectal temperature in study IV. The ambient outdoor temperature in study II was measured with a reference thermometer (Fluke T524, Fluke corporation, USA) when available.

4.1.3 Software

The freeware FLIR QuickReport 2.1 (FLIR 2012) was used to process the obtained thermographic images of all studies. This software was chosen, since it could be downloaded from the internet free of charge and would therefore be accessible to any veterinarian. Moreover, the software in question was delivered with the FLIR cameras. Currently, there are also other options for processing thermographic images taken with FLIR cameras.

4.1.4 Thermographers

Three different thermographers took the thermographic images of the dogs in the camera comparison study (I). Another thermographer, a veterinarian trained in thermographic imaging (the author of the present thesis), took the thermographic images in the greyhound study (II), cat study (III) and medical treatment study (IV).

4.2 Animals

4.2.1 Dogs

A total of 49 client-owned dogs participated in study I. The dogs were recruited by means of a public call. Adult dogs with any hip joint area condition were included in the study. The dogs were aged 1–10 years, with 16 males, 25 females, five castrated males and three sterilised females. The dogs were of 26 different breeds (Table 4) with different types of hair coats and with quite a large weight range, from 9–91 kg.

Table 4 *Demographic data of the dogs included in study I.*

Breed	Dogs (n)	Age (years)*	Weight (kg)*
Akita Inu	2	4 (1, 6)	24 (23.5, 24.6)
American Cocker Spaniel	3	4 (2, 5)	10 (8.7, 12.7)
Australian Kelpie	1	6	22
Australian Shepherd	1	1	17
Border Collie	8	4 (1, 10)	18.9 (15.0, 22.9)
Border Terrie	1	8	9
Caucasian Shepherd Dog	1	6	91
Central Asian Shepherd Dog	1	6	61
Doberman Pinscher	2	5 (4, 7)	32 (31.0, 33.0)
Dogo Argentino	2	8 (8, 9)	46 (43.6, 48.6)
Finnish Lapphund	2	3 (1, 6)	15 (15.0, 15.6)
German Shepherd Dog	8	4 (1, 8)	35.8 (24.5, 46.8)
Giant Schnauzer	1	3	38
Golden Retriever	1	1	29
Hovawart	1	8	28
Labrador Retriever	3	7 (5, 10)	32 (27.8, 34.5)
Lagotto Romagnolo	1	3	12
Lancashire Heeler	1	5	11
Mixed-breed dog	1	5	33
Nova Scotia Duck-Tolling Retriever	1	9	15
Old English Sheepdog	1	2	33
Rough Collie	2	3.5 (3, 4)	30.4 (30.0, 30.9)
Samoyed	1	2	33
Smooth Collie	1	6	22
Staffordshire Bull Terrier	1	1	18
Welsh Springer Spaniel	1	7	22

*mean (minimum, maximum)

Forty-eight racing greyhounds were enrolled in study II. The dogs were between 15 months and 8 years of age and weighed, on average, 32.4 kg, with a range of 24.7–39.5 kg. The dogs had to undergo a health check performed by a veterinarian before being cleared to race. All the dogs were therefore preliminarily considered healthy since they were participating in a race. Twelve of the 47 dogs participated in the study on both days.

Eight purpose-bred neutered Beagle dogs (two females and six males) were used for study IV. The dogs were between 3 and 4 years of age. The mean weight of the dogs was 14.5 kg, with a range of 12.9–16.1 kg. They were considered healthy based on a thorough clinical examination, including complete blood count and serum chemistry, conducted before the study.

4.2.2 Cats

One hundred and four client-owned cats were enrolled in study III. The study took place at two locations, the Veterinary Teaching Hospital at the University of Helsinki and a privately-owned cat clinic (CatVet) in Helsinki. The cats studied at the Veterinary Teaching Hospital were invited to the hospital for the study. The cats studied at the private cat clinic were regular patients of the clinic – for example, patients with mild gastrointestinal problems, dental problems or a suspected locomotor problem. Severely ill patients with advanced systemic disease were excluded from the study. One long-haired cat was also excluded due to incomplete data on the patient. All remaining cats (n=103) were included (Table 5).

Table 5 *Demographic data of the cats included in study III.*

Breed	Cats (n)	Age (years)*	Weight (kg)*
Abyssinian	7	1.8 (0.33, 4.0)	2.9 (1.7, 3.5)
Bengal	4	7.0 (5.0, 9.0)	5.8 (4.9, 7.5)
Birman	1	21	3.0
Burmese	3	8.3 (5.0, 14)	5.7 (4.0, 7.3)
Devon Rex	2	2.3 (2.0, 2.5)	3.8 (3.8, 3.8)
Domestic Longhair	6	9.5 (5.0, 15)	4.5 (3.4, 5.3)
Domestic Shorthair	44	7.7 (0.2, 16)	5.0 (0.9, 6.6)
European Shorthair	2	11 (4.5, 17)	5.0 (4.9, 5.1)
Korat	4	2.4 (0.8, 4.0)	4.5 (4.0, 5.4)
Maine Coon	8	1.7 (0.5, 5.5)	5.2 (4.3, 7.4)
Norwegian Forest Cat	3	10 (6.0, 17)	5.7 (4.4, 6.5)
Oriental Shorthair	6	6.2 (1.0, 17.5)	4.1 (3.7, 4.9)
Persian	1	6.0	3.3
Ragdoll	7	4.7 (1.0, 12)	5.0 (3.6, 7.1)
Seychellois Shorthair	2	1.0 (1.0, 1.0)	3.9 (3.8, 3.8)
Siamese	3	10 (2, 18)	4.1 (3.1, 5.3)

*mean (minimum, maximum)

4.3 Study settings

4.3.1 Surroundings for the greyhound study (II)

Since this study was conducted outdoors, the ambient temperature was measured with a reference thermometer. During the race in July 2011, this thermometer was not available to us, and the ambient temperature was measured from the thermal images by determining the coldest spots in the background temperatures in the four images taken from a specific dog, after which the warmest of these cold spots was selected for calculations. The calculated average ambient temperature on the first race day in July was 18.3°C (13.1–23.3°C) and on the second race day in September 15.7°C (14.5–16.1°C).

The thermographic images of the greyhounds (study II) were taken at a racetrack in Turku, Finland. The track in Turku is 475 metres long and has a curve diameter of 104 metres. The dogs were racing four different distances: 325, 495, 560 and 785 metres. For the 325-metre sprint, there were a total of 27 runs, and for the 495-, 560- and 785-metre sprints, 17, 9 and 6 runs, respectively. The dogs raced in an anticlockwise direction.

4.3.2 Questionnaire for cat owners (III)

Owners filled in the questionnaire concerning their cat's behaviour twice (30 minutes apart), before and after thermographic imaging, in order to test the repeatability of the questionnaire as a part of study III.

The questionnaire was compiled by choosing seventeen clinically significant questions from the original pain questionnaire by Zamprogno et al. (2010). The questions were then translated into Finnish from English. The chosen questions were piloted with cat owners after the translation (n=9) and repeated (n=5) after corrections. The original questions were modified according to the translation and the feedback from the pilot.

All seventeen questions had five alternative answers out of which the owner had to choose the one that was right concerning his or her cat. Points from the chosen 17 answers were then summed up, the minimum sum being 17 and the maximum 85. The owners' opinion of whether their cat was in any pain (none [1], occasionally [2], sometimes [3], often [4], constantly [5]) was also inquired about as a separate question at the end of the questionnaire.

4.3.3 Palpation of the cats to detect signs of possible pain (III)

In the cat study, those cats who tolerated it were also palpated before the thermographic imaging. The palpation was performed by one experienced veterinarian who was blinded to the history of the cat. The results of the palpation (spine, joints, muscles), including even minor reactions to the palpation as well as wasted muscles and trigger points in the muscles were scored by the examiner. The scoring for the palpation findings ranged from 1 to 5, 1 being normal.

4.3.4 Medication protocol for medical treatment study (IV)

All dogs received four different treatments in a randomized crossover design, with a minimum of 14 days' washout period between each treatment. The drugs used in the treatments in this study were medetomidine (Dorbene 1 mg mL^{-1}, laboratories Syva S.A., León, Spain), butorphanol (Butordol 10 mg mL^{-1}, Intervet International B.V: Boxmeer, Netherlands) and MK-467 (Merck, Sharpe & Dohme, PA, USA). Before administration, 10 mg of MK-467 was solubilised into 1 mL of saline.

The treatments consisted of the two different combinations: medetomidine 20 µg kg^{-1} and butorphanol 0.1 mg kg^{-1} (MB) with or without MK-467 500 µg kg^{-1} (MK). Both combinations were given iv and im as separate treatments.

4.4 Thermographic imaging

The emissivity of one was set for the imaging in all studies prior to initiating the imaging, since the emissivity of different-breed dogs and cats has not yet been determined.

4.4.1 Thermographic imaging in awake animals

The test animals, dogs in the camera comparison study (I) and cats in study III, were allowed to acclimatize to the room temperature for approximately 15 minutes (cats) or 30 minutes (dogs) before imaging. In the camera comparison study (I), the dogs were allowed to walk around in the examination area. The owners were instructed to remove harnesses and coats before arriving at the study location. Owners were not allowed to touch the animal during the acclimatization period before imaging. In the greyhound study (II), the dogs were imaged outside before the veterinary check-up with no shelter from the wind or sun due to the lack of a covered space in the area, and again within 5 minutes after the race in a large tent set up beside the racetrack near the gate where the racing dogs exited after the race.

During thermographic imaging, the owners and the thermographer were only allowed to touch the head or the abdomen of the dog or cat if necessary, not the torso or limbs.

The dogs in the camera comparison study (I) were positioned in a symmetrical standing position. The dogs were examined in a room with a steady ambient temperature. The specific ROI was the hip area. The thermographic images included the area from the last lumbar vertebrae to the first coccygeal vertebrae at a minimum. The thermographic images were taken by the thermographer with all three cameras, one image per camera. Immediately after the first three thermographic images were taken, the procedure was repeated by another thermographer. Out of the three thermographers, two were randomly selected to take images of each dog. The thermographic images were taken from a distance of 60 centimetres to simulate the clinical setting where the space around the patient could be limited (Figure 2). All three cameras were focused before taking the thermographic images (Figure 3).

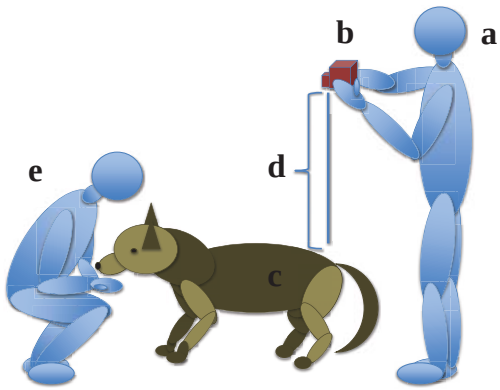

Figure 2 *Schematic presentation of the setting for thermographic imaging in the thermal camera comparison study (I). Thermographer (a) standing behind the dog taking a thermographic image with the thermal camera (b) of the dog's (c) lumbar area from a distance of 60 cm (d). Owner (e) is holding the dog from the front*

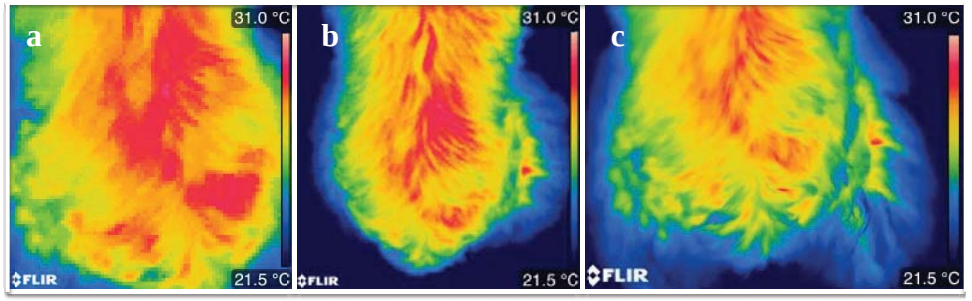

Figure 3 *Thermographic images from the lumbar area of a dog (American Cocker Spaniel) with different cameras: a) i5, 80 x 80 pixels; b) b60, 180 x 180 pixels; and c) T425, 320 x 240 pixels.*

In study II, the thermographic images of the racing greyhounds were taken from the caudal part of the dog, one distally and one proximally (Figure 4) before and after the race. This area was chosen to be the ROI, since the hind leg area is the least likely to be affected by the sun and is the most affected during the race.

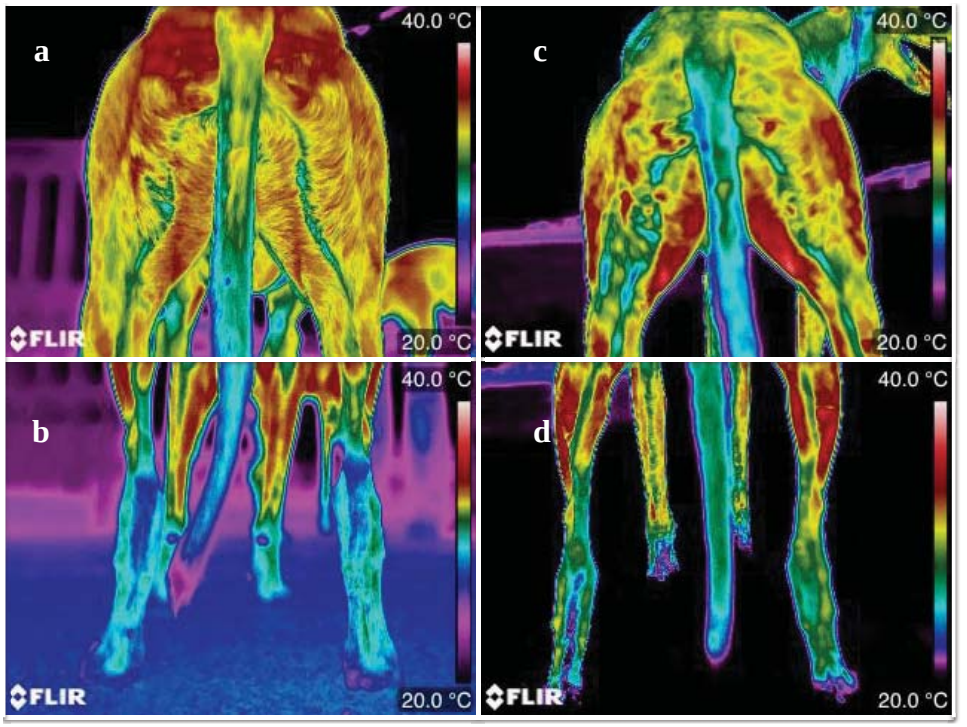

Figure 4 *Thermographic images of a racing greyhound before (a and b) and after (c and d) the race (study II).*

The cat study (III) allowed the felines to move freely in the examination room during imaging. If a cat was very shy or frightened, it was allowed to stay in the carrier box with the lid off. Five to six thermographic images were taken of each cat. Images were taken from the dorsal view as well

as from left and right lateral views and the cranial and caudal view, in addition to paw prints if possible (Figure 5). When taking thermographic images of the cats staying in their carrier boxes, a minimum of three images were taken, the dorsal view and the left and right lateral views. All thermographic images of the cats were taken before palpation.

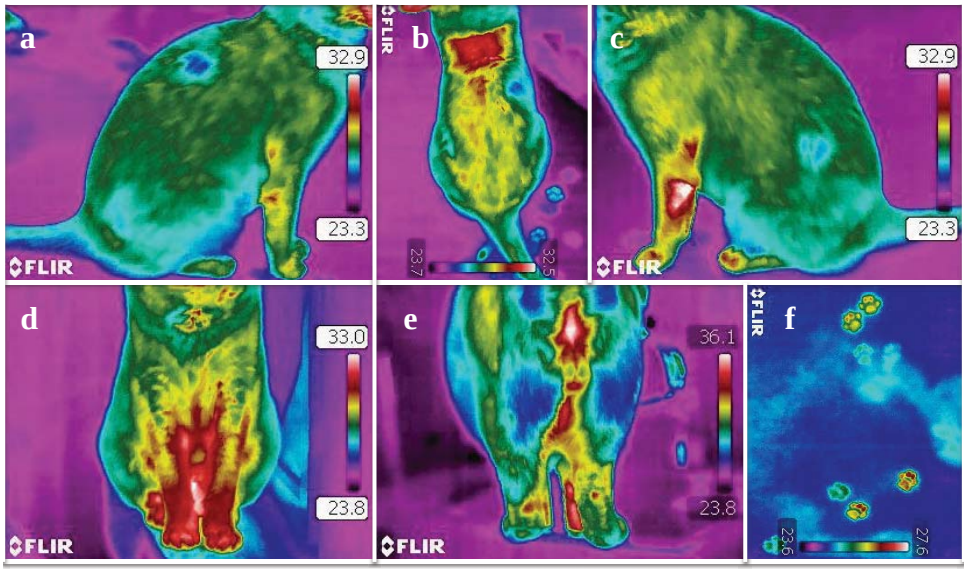

Figure 5 *Thermographic images of a clinically healthy Bengal cat (study III) a) from the right lateral side of the cat sitting down, b) from the back (the most caudal parts are seen at the bottom of the picture) of the cat sitting, c) down from the left lateral side of the cat sitting down (hairless area in the front leg appears white), d) from the front of the cat sitting down, e) from the behind, and f) paw prints from the floor where the cat had remained stationary.*

4.4.2 Thermographic imaging on medically treated animals

In the medical treatment study on dogs (IV), the canines were placed in lateral recumbency on a mattress made of insulating material, without any covering blankets, in an ambient room temperature of 21 ^{O}C during the study.

The sole of the foot in the upper hind leg of the recumbent dog was the ROI in this study. The hind leg was positioned on a holder made of the insulation mattress material to minimise the effects of the surrounding table or heat from a human hand holding the leg (Figure 6). Thermographic images and rectal temperatures were taken before administering the treatment (baseline) and repeated 3, 10, 20, 30, 45 and 60 minutes after the treatment.

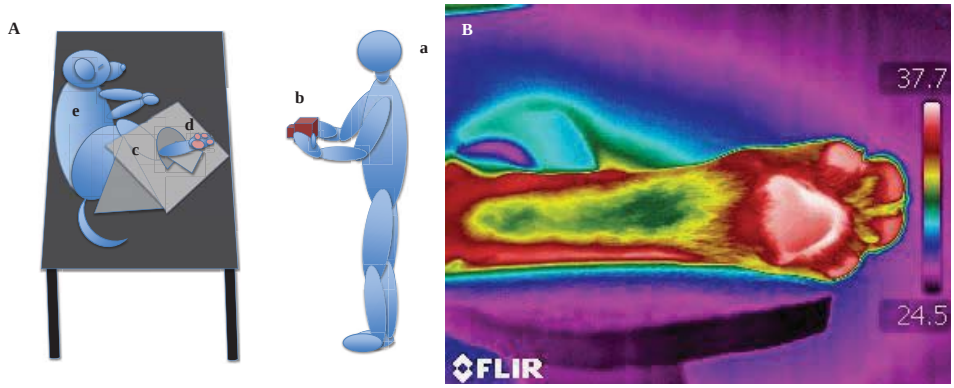

Figure 6 *A) A thermographer (a) taking an image with a thermal camera (b). Holder (c) made of insulating mattress holding the dog's upper hind leg (d). The dog lying in lateral recumbency on an insulating mattress. B) Thermographic image of the dog's foot pads.*

4.5 Interpretation of the thermographic images

Data (thermographic images) from each camera were analysed with the freeware FLIR QuickReport 2.1 (FLIR 2012). For the interpretation of the thermographic images in study I, the colour palette Rainbow was used for viewing and analysing the images. This colour palette was chosen since all three cameras (FLIR i5, b60 and T425) in study I could be set to this palette. To minimise the possible changes in the image layout during analysis, all cameras were also set to a fixed temperature interval. In studies II–IV, the colour palette High Rainbow was used.

The analysis in study I was performed by three different trained interpreters for all cameras separately and independently. In studies II–IV, one trained veterinarian analysed the images. All interpreters were blinded to the dogs' or cats' history or status during the image analysis process in all of the studies.

The ROI in study I was the anatomical hip joint area. Mean temperatures in both hips were evaluated with the software (FLIR QuickReport 2.1) by placing temperature boxes of equal size onto the ROI in the thermographic image (Figure 7).

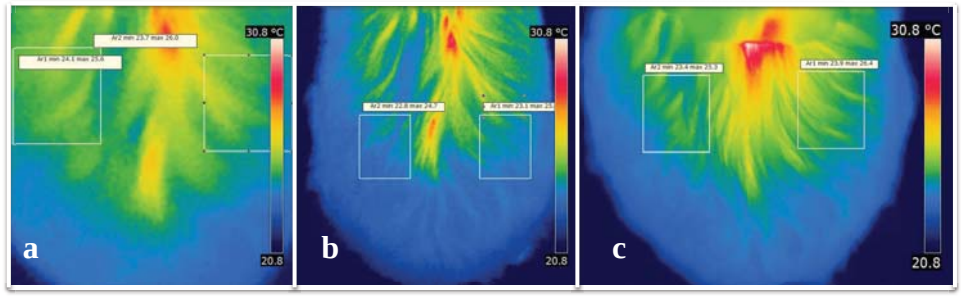

Figure 7 *Thermographic images from the lumbar area with measurements using rectangles for mean temperature with three different thermal cameras: a) i5, b) b60 and c) T425 (study I).*

In the greyhound study (II), four superficial temperature measurement points were selected: the *Tendo calcaneus, Musculus gastrocnemius, Musculus gracilis* and *Musculus biceps femoris*

portio caudalis. The thermal symmetry between the left and right leg was studied from the thermographic images by means of calculating the superficial temperature differences of the selected measurement point before and after the race (Figure 8). The measurement sites were chosen because of the region of interest is affected by physical exercise and they are easily accessed and seen with thermal imaging.

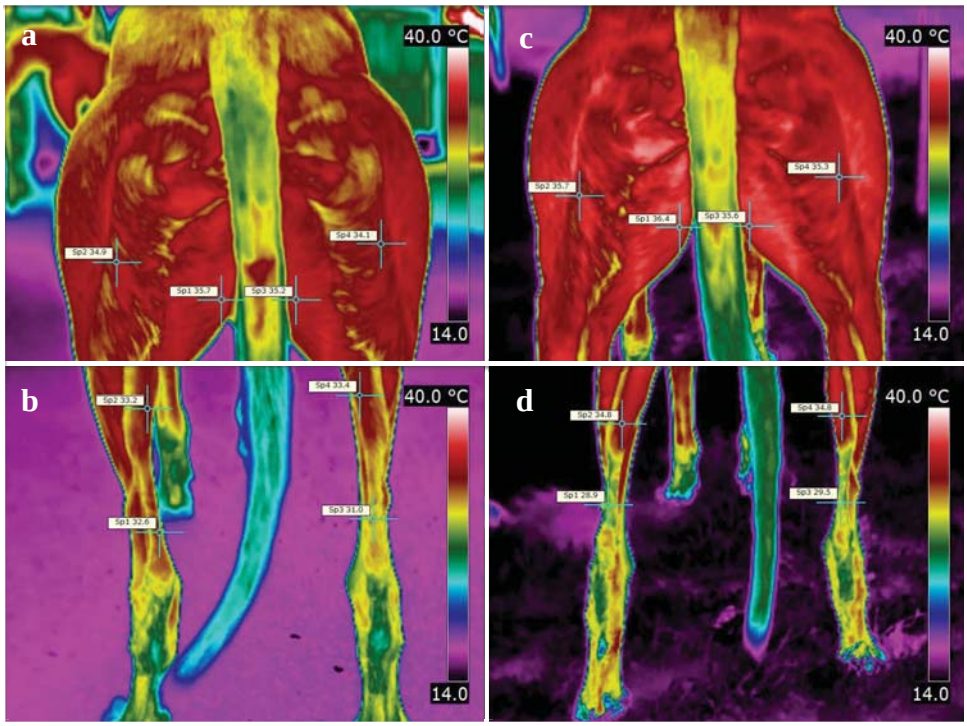

Figure 8 *Thermographic images from behind a greyhound before (a and b) and after (c and d) the race (study II).*

In the cat study (III), temperature differences between the sides (left and right: lateral view and dorsal view, etc.) of the cats were studied. Temperature differences between the sides were scored from 1 to 3 – 1 was considered normal with no findings, 2 was a mildly abnormal temperature finding (≥ 0.5 °C difference but less than 1 °C), and 3 was a temperature difference of ≥ 1 °C. Clinically abnormal cold or hot areas over the back without differences between the sides within the same individual cat were also noted. Figure 9 demonstrates the temperature measurement method for cats.

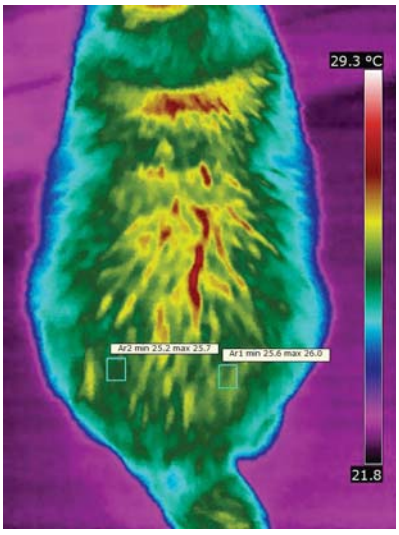

Figure 9 *A thermographic image from the dorsal view of a cat (study III) that is sitting down with the tail at the bottom of the image. Temperature measurements were taken from the hip area.*

In study IV, two different temperatures were measured from the soles of the sedated dogs' feet: DFT and MFT. Temperatures of the MFT and DFT were recorded for analysis. The MFT (the fourth or fifth) was selected for analysis depending on which foot pad was pointing directly to the camera (Figure 10).

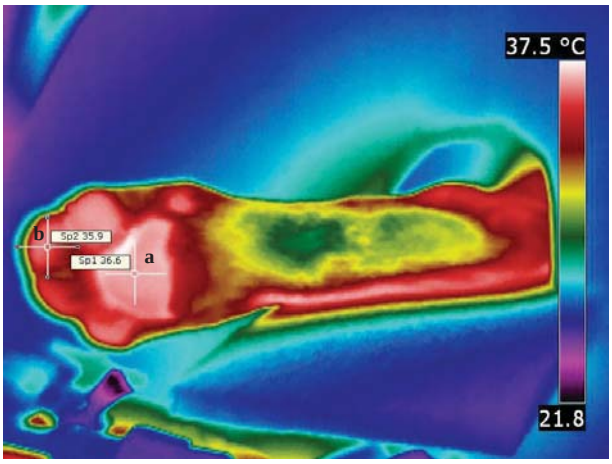

Figure 10 *A thermographic image of the sole of a dog's foot (study IV). Temperature measurements taken from the MFT (a) and the DFT (third digital foot pad) (b).*

4.6 Statistical methods

A one-arm cross-sectional experimental design was used in study I for the comparison of thermographic cameras. The analysed data included values from all three cameras, two thermographers and three interpreters for both the left and right hip joint area of each dog. The random variation caused by the interpreters and thermographers was assessed. Random effects models were selected because the different thermographers and interpreters are assumed to create only random variation to the data; in other words, the mean differences between thermographers and interpreters are assumed to be zero. The random effects models were also constructed separately for the different cameras and for the left and right legs. Estimates and 95% CIs were provided for all of the random effects models.

Another method to evaluate the importance of the differences between interpreters and thermographers in study I was also applied. A repeatability statistic between interpreters and thermographers was calculated with one-way ANOVA where the effect of the dog was used as a fixed effect. In the used models, the within-group variation describes the variation between interpreters and thermographers, respectively. The repeatability statistics were calculated based on the within-group and between-group mean squares of the ANOVA models.

In study II, descriptive statistics were constructed for superficial temperatures in greyhounds based on the thermographic images by time point (before vs. after race), leg, day and distance for each site (*Tendo calcaneus, Musculus gastrocnemius, Musculus gracilis* and *Musculus biceps femoris portio caudalis*). The differences between the time points, legs and days in the superficial temperatures based on the thermographic images were evaluated for each measurement point separately with analysis of variance for repeated measures. The relationship between the difference between the before-race and after-race superficial temperatures was assessed with ANOVA models for each measurement point separately.

In study III, the scores of palpation findings on the cats were compared to the questionnaire and thermographic images as well as to the results of the questionnaire and the owner's opinion of assumed pain. From the answers regarding assumed pain, one was considered normal, 2 and 3 were considered moderate, and 4 and 5 were considered severe pain. The pain question scores were merged (1=1, 2-3=2, 4-5=3) for statistical analysis. The question concerning the owner's opinion of assumed pain was analysed separately and compared to the questionnaire as well as the thermographic images and palpation findings.

The agreement between the owners' evaluation of the cats' pain, the palpation results and the thermographic image results were assessed pair-wise with the aid of Weighted Kappa coefficients using Cicchetti-Allison's (Cicchetti & Allison 1971) weights. In this model, kappa values of 0.01–0.20 were considered as slight agreement, 0.21–0.40 as fair agreement, 0.41–0.60 as moderate agreement, 0.61–0.80 as substantial agreement and 0.81–0.99 as almost perfect agreement (Viera & Garrett 2005).

The relationship between the questionnaire, palpation and thermographic image results was assessed with one-way ANOVA models. Least square means from these two models were used in the determination of reference limits for questionnaire scores.

The quality of the determined reference limits for pain was assessed in two different ways. Sensitivity and specificity were calculated for dichotomized classification variables (pain/no pain). This means of analysis provided information on how well the classification based on the pain questionnaire identified pain-free cats and those in pain, assuming that the palpation or thermographic image results were correct. The other method applied in assessing the reference limits did not assume that the palpation and thermographic image results were correct and used the original three-category classification from the questionnaire. The ratios of consistency and their 95% CIs were calculated for each of the categories (no pain, probable pain, distinct pain).

The random variation caused by refilling the questionnaire was evaluated in two different ways, with a random effects model and an ANOVA model. The response in the models was the pain score acquired from the questionnaire by summing up the answers. In the case of a specific question being unanswered the first or second time of taking the questionnaire, the specific question was also considered missing (for the specific cat) the other time in order to make the sums comparable. Repeatability statistics between the two time points were calculated from the ANOVA model.

In study IV, the differences in the change from baseline values between the medical treatments and routes of administration were assessed with repeated measures analysis of covariance models. The models consisted of a baseline covariate, the main effects of treatment, the period and time point of measurement as well as two-way interactions of period*time point and treatment*time point as fixed effects and the main effect of dog, in addition to two-way interaction terms of period*dog and time point*dog as random effects. Ninety-five percent CIs and p-values were calculated for the estimated group differences. A p-value of < 0.05 was considered statistically significant.

All statistical analyses for studies I and IV were performed using the SAS® System for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA) and for studies II and III SAS® System for Windows, version 9.3 (SAS Institute Inc., Cary, NC, USA).

4.7 Ethical committee approval and owners' consent

Written informed consent for participation in the study was requested from all dog and cat owners before the enrolment of their pets in the studies (I–III), and the study protocols were approved by the Ethical Committee of the Viikki Campus, University of Helsinki (7/2009, dated 14.1.2010).

The medical treatment study (IV) protocol was approved by the National Animal Experimentation Board of Finland (ESAVI-2010-07734/Ym-23).

5 Results

5.1 Comparison of different resolution thermal cameras (I)

Three different resolution cameras were compared using between-interpreter variation and between-thermographer variation as indicators for the comparison. The resolutions were 80x80 pixels, 180x180 pixels and 320x240 pixels for the cameras i5, b60 and T425, respectively. The between-interpreter variation was small with all the cameras used. The estimates with 95% CIs were 0.04 (0.03–0.06) with camera T425, 0.05 (0.04–0.07) with camera b60 and 0.13 (0.1–0.17) with camera i5. When using the higher-resolution cameras T425 and b60, the variation between thermographers also seemed to be minor. The estimates with 95% CIs were 0.09 (0.06–0.14) for T425 and 0.11 (0.07–0.17) for b60. With camera i5, the variation between thermographers was notably higher, almost 0.4 (0.27–0.6).

The variation between dogs varied from 4.6 to 5.2 depending on the fitted model, being much higher than the variation between thermographers. These previous estimates, excluding between-thermographer variation with camera i5, seem negligible compared to the variation caused by dogs.

5.2 Repeatability of the thermographic images (I)

The resolution of the thermal camera was found to be an influencing factor for the repeatability of the images. The repeatability between the persons taking the thermographic images (thermographers) and those who evaluated the images (interpreters) was the highest with the highest-resolution camera, T425. The repeatability between thermographers was 0.927, 0.976 and 0.981 for cameras i5, b60 and T425, respectively. The repeatability between interpreters was 0.974, 0.989 and 0.991 for cameras i5, b60 and T425, respectively.

5.3 Animals in the studies

5.3.1 Dogs

5.2.1.1 Hair coat

Although the dogs in the camera comparison study (I) represented multiple breeds with different kinds of hair coats, the impact of hair coat on within-dog variation was not considerable in the thermographic images. The hair coats varied from very short with no underlying fur to very thick and long hair. In addition, one dog with curly hair participated in study I. Figure 11 shows thermographic images of different breed dogs in study I.

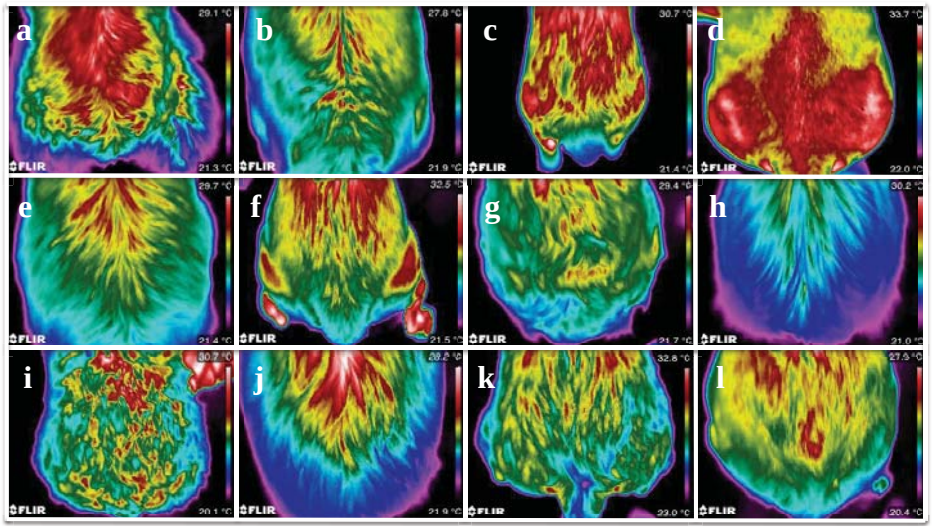

Figure 11 *Thermographic images of the lumbar area of different breed dogs: a) American Cocker Spaniel, b) Akita Inu, c) Border Terrier, d) Dogo Argentino, e) Hovawart, f) Australian Kelpie, g) Golden Retriever, h) Finnish Lapphund, i) Lagotto Romagnolo, j) Rough Collie, k) Giant Schnauzer and l) German Shepherd.*

5.3.1.2 Effects of physical excercise

In the study on racing greyhounds (II), the superficial temperatures were significantly higher after all raced distances than at baseline in one measurement point, namely the *Musculus gastrocnemius*. The magnitude of the differences before and after the race varied depending on the measurement point (*Tendo calcaneus*, *Musculus biceps femoris portio caudalis* and *Musculus gracilis*). The superficial temperature differences in all measurement points between the left and right leg after the race varied from 0.0 to 4.0 degrees. However, neither leg (left or right) was systematically warmer than the other. No clinical signs of obvious injuries were detected during or after the race by the veterinarian responsible for the racing dogs.

Ambient temperature affected the surface temperature of the dogs. A significant difference was detected between the two race days – warmer surface temperatures were observed on the race day in July than on the one in September with respect to all but one measurement point after the 325- and 495-metre sprints, the exception being the measurement point of *Musculus biceps femoris portio caudalis* after the 325-meter sprint. Details are presented in original article II.

5.3.1.3 The effect of medical treatment on thermographic images in dogs

In study IV, the superficial temperature difference between treatments was approximately 0.5 degrees, although the temperature differences between treatments did not arise immediately after the drug administration. With the iv and im routes of MB, the MFT declined immediately after administration and, at 10 minutes, began to slowly increase towards baseline. The MFT with the combination of MB and MK was higher (average 3.5 degrees) than with MB alone.

The temperatures of DFTs were similar to those of MFTs. Figure 12 depicts the temperature changes at all the measured time points with the different treatments.

The rectal temperature was lower with the MB and MK combination than with MB alone regardless of the route of administration.

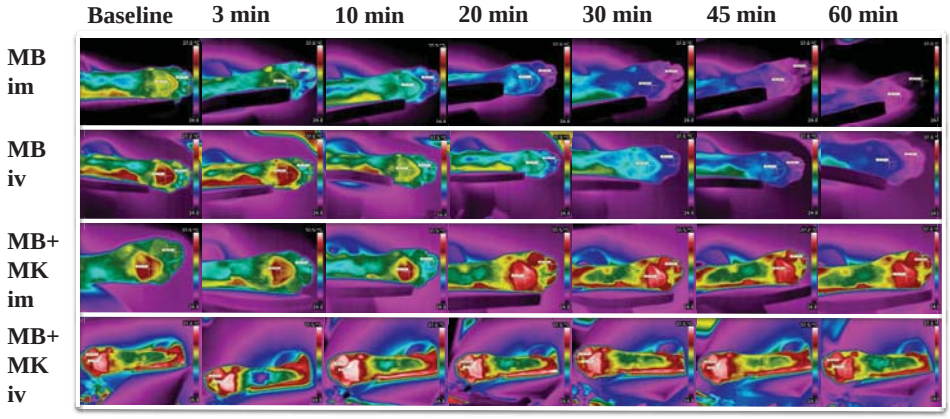

Figure 12 *Thermographic images of dogs' foot pads (MFT and DFT) with all treatments at different measurement points.*

5.3.2 Cats

5.3.2.1 Hair coat

The enrolled cats represented 16 breeds, including long-haired and short-haired ones (III). All the cats that did not have short hair were considered to be long-haired cats, the length of the hair coat varying from moderate to long. Figure 13 shows thermographic images of different breed cats in study III.

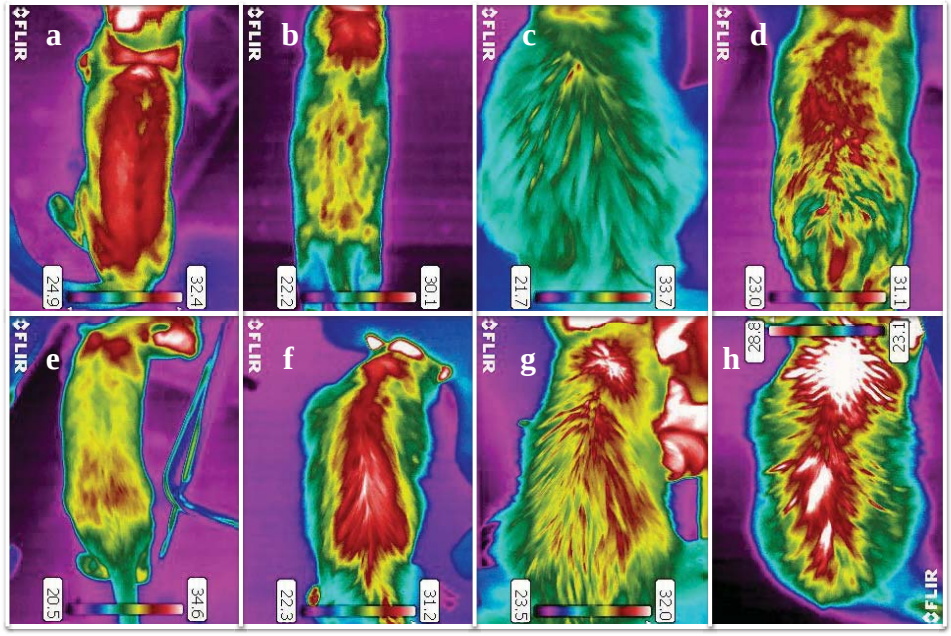

Figure 13 Thermographic images of the lumbar area of different breed cats: a) Abyssinian, b) Bengal, c) Domestic Longhair, d) Domestic Shorthair, e) Korat, f) Maine Coon, g) Norwegian Forest Cat, and h) Ragdoll.

5.3.2.2 Palpation

A total of 95 out of 103 cats were clinically palpated. Very frightened cats (n=8) were not handled at all, although the thermographic images were taken from a set distance. Of the palpated cats, 47 (49%) showed signs of discomfort during the palpation, which was interpreted as possible pain in the palpated area. All the cats in study III were able to move without obvious difficulty or lameness.

5.3.2.3 Thermographic imaging of the cats

Examples of irregular heat patterns are shown in Figure 14. Fifty-six percent of the cats in the study (58 out of 103) showed irregular heat patterns in their thermographic images, either on the torso or on the torso and paw prints. Out of the 47 cats that showed signs of discomfort during palpation, 39 had irregular heat patterns in their thermographic images, which is 59% of all cats in the study. Thermographic imaging was possible with all cats in study III without any resistance from the cats.

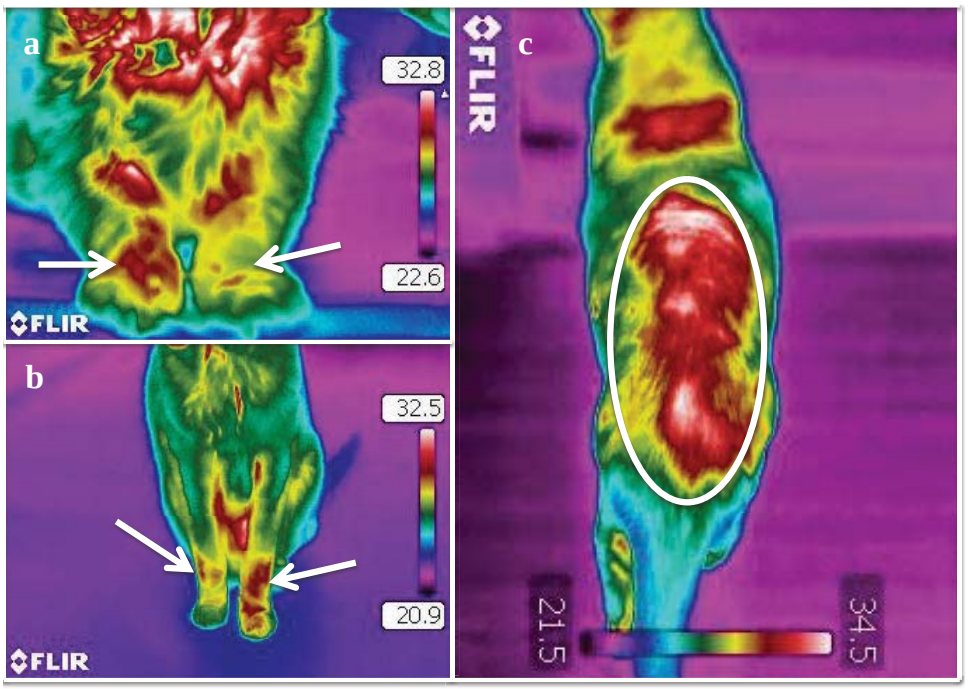

Figure 14 *Thermographic images of cats with suspected painful processes: a) uneven heat pattern in carpal area, with right carpal area markedly warmer (arrows); b) uneven heat pattern in the front legs, with left distal limb area markedly warmer (arrows); and c) uneven heat pattern in the back of the cat (oval).*

5.3.2.4 Questionnaire

In the cat study (III), the questionnaire was filled in twice in order to assess repeatability. The repeatability of the questionnaire was 0.97, indicating that the variation between repeats represented a very small part of the total variation.

Twenty-two cats were reported by their owners to have some pain sometimes (19%) or constantly (2%). Only four cats had previously diagnosed osteoarthritis (4%), and two were suspected to have osteoarthritis (2%). Five cats were on oral glucosamine sulphate with or without chondroitin sulphate, one cat received acupuncture regularly, but none of the cats in the study were on non-steroidal anti-inflammatory drugs at the time of the study.

5.3.2.5 Comparison of thermographic images, palpation, questionnaire and owners' estimate of pain response in cats

Two different cut-off values for pain questionnaire scores were selected to classify cats as being in pain or pain-free, based on the fitted ANOVA models. In addition, values classifying pain were classified into three categories according to the questionnaire, and two different limits were assessed.

The agreement between palpation and thermographic imaging of the cats was moderate (0.428). The agreement between the owner's evaluation and thermographic image results or palpation results was fair.

Overall, the proportions of agreement between thermographic images or the pain questionnaire, or between the pain questionnaire and palpation results, were quite low, especially for the lowest two of the three categories (probable pain and distinct pain) indicating pain. Detailed information is presented in original article III.

6 Discussion

This thesis was intended to identify the thermographic camera best suited for clinical veterinary use, and to determine the effects of different thermographers on the thermographic images as well as the influence of different interpreters on the received results. The method of thermographic imaging was also utilised in distinct clinical settings.

6.1 Thermal cameras and thermographic images

6.1.1 Thermal cameras

In the first study (I), we examined the reliability of thermographic imaging by assessing the variation and repeatability of different resolution cameras, different thermographers and different interpreters, using basic software for analysing the images. The resolution of the camera was discovered to have a considerable impact on the variation between thermographers. The higher the resolution of the camera, the higher was the repeatability between thermographers. In the lowest-resolution camera, FLIR i5 with a resolution of 80 x 80 pixels, the repeatability was not directly correlative. When using the higher-resolution cameras b60 and T425 with resolutions of 180 x 180 pixels and 320 x 240 pixels, respectively, the repeatability between thermographers was equal.

The impact of the resolution of the camera on repeatability is understandable since small changes in the camera lens angle in relation to the object may influence the amount of pixels attained from the ROI (Watmough et al. 1970). Even though the thermographic images in study I were taken holding the camera lens perpendicularly (90°C) to the ROI, the curvature nature of an animal may have led to false interpretations due to fewer pixels per inspected area. Therefore, in a low-resolution camera such as i5, small changes in the image cannot be interpreted and placed in perspective due to the lack of resolution and the small amount of pixels in the ROI. The present results from study I indicate that a resolution of 180 by 180 pixels, such as in b60, is enough to give repeatable results. Higher resolution in a camera also means that smaller differences in temperature can be detected. However, in spite of the problems, a low-resolution camera (80 x 60 pixels) has been used for scientific purposes (Levet et al. 2009). Even if they have been used previously in research settings, clinical settings usually cannot be standardized in a similar manner and good results cannot be anticipated. Currently, the available cameras and their reduced price over the years have made it possible for the practitioner to use cameras with a high resolution.

6.1.2 Software

We analysed all the thermograms with the basic freeware downloadable from the internet (FLIR 2012) and supplied with the thermal camera used in the studies. After establishing in study I that different interpreters attained similar result using this basic freeware and that it could be used to interpret thermographic images, we deduced that it would be sufficient to be used in the other studies (II–IV) as well. The software used enabled the measurement of mean temperatures of rectangular shapes (Figures 7 and 9) or temperatures of small measurement points (Figures 8 and 10) from the thermographic images. We attained temperatures in the comparison of cameras (I) and in cats (III) using a rectangular shape, which has also been applied to interpret the thermographic images in other studies (Park et al. 2007; Bowers et al. 2008; Ferreira et al.

2008; Spalding et al. 2008; Wu et al. 2009; Denoble et al. 2010; Vardasca et al. 2012). Currently, new freeware, FLIR Tools (FLIR 2013), is available with the option of using a circular shape for temperature analysis. The other possibility was to use measurement points as we did in greyhounds (II) and when observing the effects of medical treatments (IV) (Figure 8 and 10). More sophisticated software with a wider spectrum of possibilities for image processing are available by either purchasing or having one custom-made, as demonstrated in several studies (Autio et al. 2007; Stewart et el. 2007; Song et al. 2007; Ferreira et al. 2008; Gold et al. 2009; de Weerd et al. 2009; Levet et al. 2009; Infernuso et al. 2010; Gazerani & Arendt-Nielsen 2011; Simoes et al. 2012; Vardasca et al. 2012; Westermann et al. 2013a).

For a beginning practitioner, software that is easily acquired, with low or no cost, and simple to use would probably be the first choice. Therefore, it is important that the usability and repeatability of such software can be ensured. We used the FLIR QuickReport 2.1 (FLIR 2012) software successfully in four studies, and the results suggest that this method could easily be applied to clinical small animal practice with relatively minor training in the use of infrared cameras and interpretation of thermographic images.

6.1.3 Hair coats of cats and dogs

The dogs and cats in studies I and III, respectively, were of varying breeds and, more importantly, had different kinds of fur. We observed that the length and thickness of hair coats differed markedly in appearance in thermal images, which may have complicated locating the ROI. This was especially challenging when the hair was very long and/or thick, since a thick hair coat is a physiological protection against excessive heat loss (Henshaw et al. 1972; see review McCafferty 2007). Moreover, a thick hair coat might prevent subtle temperature differences in the skin to be captured with thermographic imaging. Furthermore, we noticed that curly hair made the reliable estimation of the mean temperature difficult, since the heat escaped from the underlying skin in irregular patterns. In humans, the heat emitted from the body is not trapped inside insulating fur as in dogs and cats, and direct measurement of skin temperature is possible (Gazerani & Arendt-Nielsen 2011; Park et al. 2012) within the limitations of the thermal cameras.

Other method besides those which we used to recognise the anatomical structures and the ROI in thermographic images is to create an artefact to the ROI, such as a silver sticker, as demonstrated in humans (Denoble et al. 2010). However, the sticker attachment site may change when the skin or hair coat moves. In animals, moisture has been used to create an artefact, but the method has not been published. Artefacts could interfere with the interpretation, which is why taking thermographic images before and after creating the artefact is essential (Denoble et al. 2010). Furthermore, with most currently available thermographic cameras, it is possible to take simultaneous digital photograph from the subject during imaging, making it easier to recognise the ROI.

6.2 Effects of exercise

In the greyhound study (II), we evaluated the superficial temperature before and after the race. The superficial temperatures evaluated by means of thermographic images were significantly higher on the *Musculus gastrocnemius* after the race than before the race for all the raced distances. This change in superficial temperature was predictable since the muscle work generated a considerable amount of heat during the race.

Racing greyhounds have a lean body type with a small amount of body fat (Zaldívar-López et al. 2011), and the hair coat is thin throughout and, in those areas (abdomen, hind legs) where the wind effect during running is presumably the largest, very thin or lacking completely. The body and hair type of racing greyhounds presumably contribute to the heat loss, since the dogs expressed no signs of greater discomfort on the warmer race day in July when compared to the colder race day in September. However, neither of the days was extremely hot. For hundreds of years, racing greyhounds have been bred for running, and their physiology has been adapted to deal with heat generated during running. Since thermographic imaging is able to detect changes in temperature due to heat loss (Varjú et al. 2004; Vianna & Carrive 2004; Stewart et al. 2007), it may become a valuable tool in monitoring animals' heat balance status, as suggested in study II.

A marked finding in study II was that the temperature difference between the legs after the race was up to 4.0°C without any obvious signs of injury, although in studies conducted on humans without exercise, a less than 1°C temperature difference between the sides has been considered abnormal (Uematsu et al. 1988a; Ammer et al. 2001; Hakgüder et al. 2003; Lee et al. 2007; Simoes et al. 2012; Vardasca et al. 2012). Immediately after the race, the notable temperature differences detected with thermographic imaging were probably due to physiological changes generated by muscle work and blood flow due to the asymmetrical use of the musculature when running on an oval-shaped race tract. Unilateral injuries can also be seen as superficial temperature differences between the left and right sides in thermographic images (see review Turner 2001; Vardasca et al. 2012). Previously, thermographic imaging has been used before and after the exercise to detect subclinical osteoarthritis in horses (Vaden et al. 1980). The characteristics of the racetracks, raced distance and speed of the dog have been demonstrated to affect the rate of injuries (Sicard et al. 1999). In our material, however, no systematic asymmetry was detected between the left and right sides of the dogs. Our findings suggest that thermographic imaging might not be a useful tool in determining the extent of an injury immediately after a race. Injury detection with thermographic imaging could be more productive after the cooling of the dog after the race. In addition, a concurrent clinical examination when performing thermographic imaging is essential for determining the extent of possible injury detected with thermographic imaging.

6.3 Detection of painful conditions with thermographic imaging

It has been previously determined in dogs that the visual analogue scale and Helsinki chronic pain index correlate with pain (Hielm-Björkman et al. 2009, 2011). A corresponding questionnaire for cat owners concerning their pet's behaviour correlated with DJD-associated pain (Zamprogno et al. 2010). Herein, the questions (Zamprogno et al. 2010) were translated and used as a questionnaire for assessing pain in cats (III). We chose this questionnaire since we wanted to observe potentially painful conditions due to chronic processes. However, in our study, the association between thermographic findings in the cats and the questionnaire was not conclusive, which can be explained by the fact that not all changes in thermographic images are results of joint problems and not all chronic joint problems, even the severe ones, are physiologically active at all times (Varjú et al. 2004). When cats with suspected DJD were examined using an orthopaedic examination, goniometric measurements and radiography, the association between the modalities was not always clear (Lascelles et al. 2012). Since no gold standard for diagnosing mild pain in cats currently exists, palpation and manual manipulation are often the only methods to detect pain in clinical practice (see review Kerwin 2012). In species

with a very subtle behavioural expression of pain, such as cats (see reviews Hellyer & Gaynor 1998; Beale 2005), thermographic imaging could be a very useful diagnostic tool.

In the present cat study (III), no other diagnostic modalities, such as radiological examination or CT, were used since our aim was to study the thermographic method with cats in basic veterinary clinic conditions. Furthermore, some of the potentially painful cases, such as osteoarthritic pain in cats, cannot be detected in radiological examinations (Clarke & Bennet 2006; Lascelles et al. 2012).

When using pain scales to evaluate pain in animals, the person performing the evaluation should be educated to recognise the changes in behaviour, as demonstrated in dogs (Hielm-Björkman et al. 2009, 2011) and in study III on cats. Our results from the questionnaire, palpation and thermographic imaging suggest that owners not trained in evaluating their cats' pain missed the signs of possible pain more often than a veterinarian performing palpation and using thermographic imaging.

In study III, all the cats tolerated the thermographic imaging, but some did not tolerate the palpation. With thermographic imaging, it was possible to observe the thermal symmetry or asymmetry of the cat without handling it. Although changes in the temperature can be the result of some pathologic processes such as an inflammation in an osteoarthritic joint (Varjú et al. 2004), it can also be due to a change in weight bearing and, thus, a change in muscle activity. Thermal asymmetry in cats may result from the cat lying in the transport box with the other flank down before the thermographic imaging – therefore, in study III, cats were allowed to roam free in the examination room, when possible, to become acclimatized. Since the asymmetries can also result from artefacts, paw print thermographic images were taken from the floor when possible to minimise the possibility of incorrect interpretation of the thermal asymmetries in the images. Our study suggested that, under controlled conditions, thermographic paw prints could be used to evaluate weight bearing and to assess their input as regards the other findings.

Although thermographic imaging has been applied to study and monitor the dynamics of disease activity in painful conditions such as coccygodynia in humans before and after conservative treatments (Wu et al. 2009), studies observing and monitoring painful conditions in cats have not, to the authors' knowledge, been published previously. The reason for this could be that pain in cats is still widely underestimated in general and that cats are difficult to restrain without touching them and too small for using harnesses or collars without affecting the thermographic images. However, thermographic imaging has been used successfully with dogs (Loughin & Marino 2007; Infernuso et al. 2010; see review Marino & Loughin 2010) that are also relatively small. In study III, we found the technical problems concerning the imaging of movable targets such as cats to be relatively minor. However, taking thermographic images of cats requires rapid reactions and good mobility from the thermographer. Moreover, the cats seemed quite relaxed during study III. Therefore, the owners responded enthusiastically to the study, as has also been reported with paediatric human patients and their parents when using thermographic imaging (Saxena & Willital 2008).

When examining potentially painful conditions in cats, thermographic imaging could be used to detect temperature differences in ROIs between the sides, in addition to serving as an indicator for further clinical examinations, but, according to study III, thermographic imaging is not a tool for direct diagnosis.

6.4 The effect of vasoactive medical treatment on thermographic images

In study IV, we measured the foot pad temperatures after sedative medication (medetomidine, butorphanol and MK) in dogs. The various effects of these agents on superficial circulation were visualised with thermographic imaging.

Alpha-2-adrenergic agonists, such as medetomidine, cause vasoconstriction, which is mediated via post-synaptic alpha-2-adrenoceptors located in vascular smooth muscle, and it results in an increased systemic vascular resistance (Flacke et al. 1990). Butorphanol is an agonist-antagonist opioid, frequently combined to medetomidine to improve sedation in dogs (Ko et al. 2000; Kuo & Keegan 2004). The peripherally acting alpha-2-adrenergic antagonist MK-467 selectively blocks the alpha-2-adrenoceptors located outside the blood-brain barrier (Clineschmidt et al. 1988) and attenuates the peripherally mediated cardiovascular effects of alpha-2-agonists in dogs (Pagel et al. 1998; Enouri et al. 2008; Honkavaara et al. 2008, 2011), sheep (Bryant et al. 1998; Raekallio et al. 2010) and horses (Bryant et al. 1998; Vainionpää et al. 2013).

Study IV demonstrated that when the superficial blood flow was enhanced by vasodilation (MB with MK), the heat loss was, as expected, significantly higher and could therefore, if not treated appropriately, lead to hypothermia more easily than when using vasoconstrictive drugs (MB). Hypothermia is a well-known problem in sedated and anaesthetised animals, since they may have an impaired ability to regulate temperature and vasodilative agents can enhance cooling during drug effect (Díaz & Becker 2010). It can occur in animals sedated with medetomidine (Vainio 1989), especially when no active attempts have been made to maintain the body temperature. In study IV, the dogs were insulated from the table with a mattress, with no blankets placed on them. This did not seem to be completely adequate for keeping the rectal temperature from declining further when the animals were sedated with the combination of MB and MK as opposed to using MB alone.

Although infrared thermometer measurements from the human forehead are comparable to rectal temperature (De Curtis et al. 2006) and fever can be detected with thermographic imaging (Chiu et al. 2005), the same does not apply to animals due to the hair coat in the forehead area. Furthermore, the temperature measurement from an extremity, such as MFT or DFT in study IV, probably reflects the superficial peripheral temperature changes more than it does core temperature changes. In study IV, the MFT temperature rose and the RT declined with MB with MK, and both the MFT and RT declined after MB treatment, but they did not seem to be fully associated. However, being hairless, the foot pad could be considered to have a similar appearance to human skin reflecting the superficial temperature. Thermographic images of the foot pad could be usable for evaluating the temperature of the extremities and predicting heat loss from them. The results from study IV indicate that thermographic imaging could be used for further evaluation of thermoregulatory safety in medicated animals.

6.5 Limitations of the studies

As thermographic imaging has not been studied widely on small animals, no reference data is readily available. However, when comparing the temperature differences between the sides of an animal, usable information could be obtained with the currently available knowledge on thermographic imaging in animals and humans. In addition to the lack of reference data, we acknowledge the possible influence of an unidentical environment, such as clinical settings, on

the results. In our studies, we attempted to keep the circumstances as identical as possible for every subject to avoid any artefacts that could further influence the results.

In the camera comparison study (I), greyhound study (II) and cat study (III), the subjects were client-owned animals seen in clinical settings, and no absolute standardization in regard to the patients or surrounding could be achieved. Study II was performed outdoors, which made standardization even more difficult. Possible artefacts, such as direct sunlight and wind, may have severely altered the temperatures.

In the medical treatment study (IV) that was performed using purpose-bred beagles, the settings remained identical for every study subject. On the other hand, the setting in study IV did not resemble the real clinical setting, as was the case in the other studies (I–III).

When imaging animals, the image processing rate of the thermographic camera is essential. If the image processing of the camera is very slow, the animal may have time to move and the risk of a blurred image increases. This is crucial, since positioning the animals for imaging was difficult. Some of the dogs could not stand in a symmetrical position due to minor anatomical deviations or a suspected painful process (studies I and II), and most cats (study III) would not remain stationary for a very long time.

It was also possible that the ROIs used to measure mean temperatures had been chosen incorrectly, since we did not use any anatomical markers. This is probable especially with the long-haired animals in the camera comparison study (I) and cat study (III), and with animals in an asymmetrical position.

When conducting studies at different geographical locations and over different time periods with an uncooled thermal camera, the images might not be comparable when focusing on absolute temperatures. All the cameras used (FLIR i5, b60 and T425) had an accuracy of ±2°C, thus the precise temperature readings may have deviated due to the camera as well as due to the surroundings. However, when comparing temperatures between the sides using the subject as its own control, the accuracy of the camera has no effect on the results when the imaging setting is standardised.

6.6 Clinical implications in veterinary medicine and future prospects

The results of the present studies (I–IV) suggest that thermographic imaging can be utilised more extensively in clinical small animal veterinary medicine. When studying painful conditions, thermographic imaging could be used as a method for detecting temperature differences in ROIs between sides as related to possible painful processes and, therefore, as an indicator for further clinical examinations. In the future, thermographic imaging could be used more extensively when suspecting possible painful conditions in many animal species, such as cats, since this method was well tolerated by the cats and their owners (III), and pain perception in cats is recognised to be difficult (see review Hellyer & Gaynor 1998; see review Beal 2005).

Empirical use of thermographic imaging in dogs has suggested that thermographic imaging could be adopted in monitoring trauma patients during rehabilitation, such as physiotherapy, acupuncture and laser treatments; in neurologic and soft tissue injury diagnostics; in assessing gait abnormalities; and in monitoring working and competition dogs. However, the use of sedative medications affects the superficial temperature (IV), which should be taken into consideration if thermographic imaging is utilised on patients sedated for other procedures such as a radiological examination. In addition, with older veterinary patients in whom sedation

for the purposes of diagnostics may be risky, thermographic imaging could be opted for as a screening tool to indicate and focus further diagnostic examinations. Furthermore, monitoring surgical veterinary patients, especially orthopaedic ones, before and after surgery could provide valuable information about the healing process and the successfulness of the procedure.

Thermographic imaging could also be utilised for the prevention of cruelty against animals by revealing the use of local anaesthetics in animal shows and competitions. Additionally, since normal testicular temperature patterns in various animals are known (Purohit et al. 2002), thermographic imaging could expose animals with testicular or other prostheses in animal shows, thereby improving the fair and ethical treatment of show animals.

7 Conclusions

1. Our studies indicate that a thermal camera suited for veterinary imaging purposes should have a minimum resolution of 180 x180.

2. The variation introduced by trained thermographers and interpreters is negligible when evaluating thermal camera images that have been taken with cameras of sufficient resolution. In practice, the variance introduced by thermographers and interpreters of thermographic images does not affect the clinical use of thermographic imaging.

3. The freeware QuickReport 2.1 proved to be achievable for the interpretation of thermographic images obtained from dogs with proper training of the interpreter.

4. The variance of the imaging subjects (cats and dogs) was negligible. Thermographic imaging proved to be a potential method for clinical use in imaging cats and dogs.

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