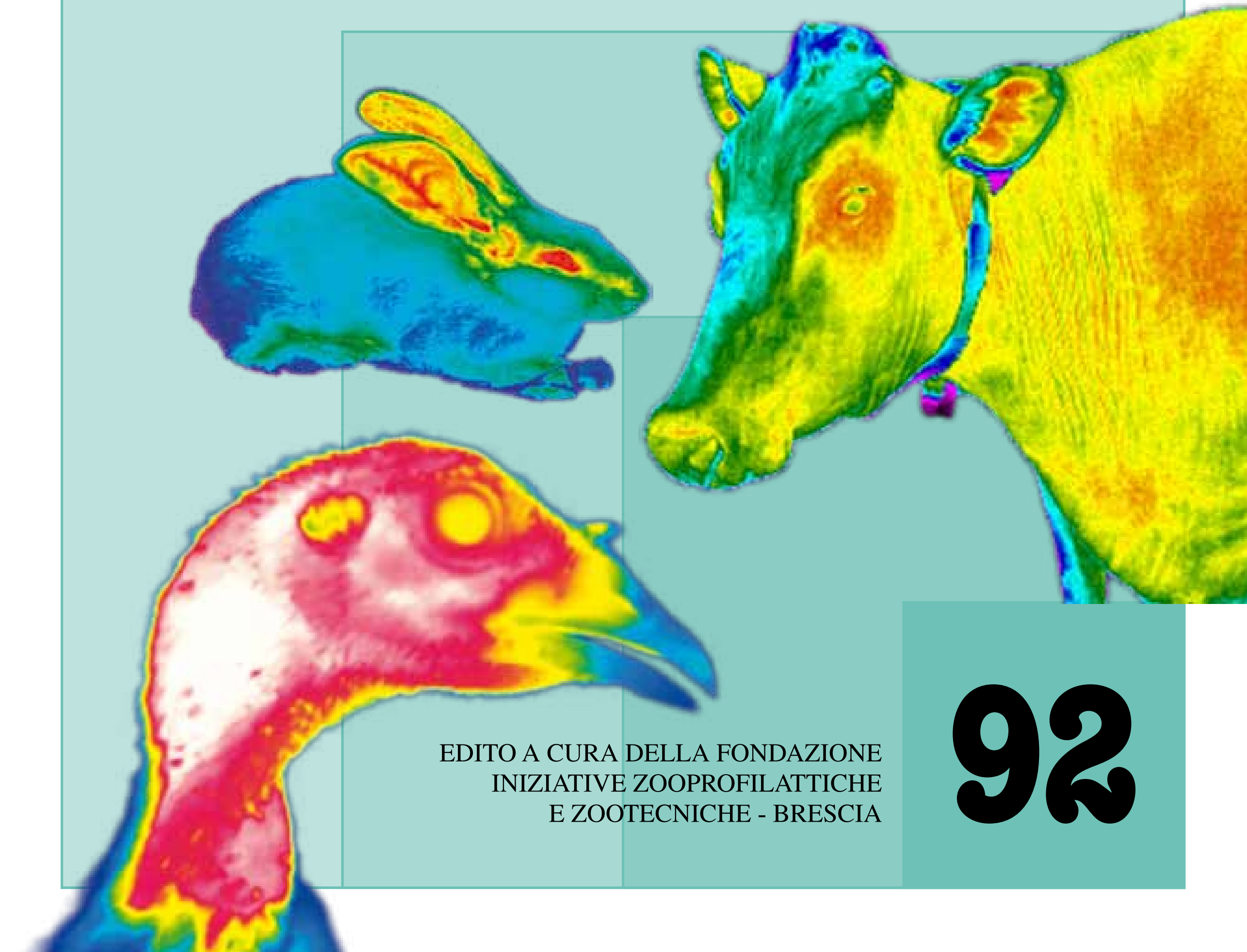
FONDAZIONE INIZIATIVE ZOOPROFILATTICHE E ZOOTECNICHE
BRESCIA

THERMOGRAPHY

CURRENT STATUS AND ADVANCES IN LIVESTOCK ANIMALS AND IN VETERINARY MEDICINE

Editors:
Fabio Luzi, Malcolm Mitchell, Leonardo Nanni Costa, Veronica Redaelli



EDITO A CURA DELLA FONDAZIONE
INIZIATIVE ZOOPROFILATTICHE
E ZOOTECNICHE - BRESCIA

92

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AND ADVANCES IN LIVESTOCK ANIMALS
AND IN VETERINARY MEDICINE

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- BRESCIA, ITALY -

Scientific Director: Prof. E. LODETTI

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THE EDITORS

The Editors would like to express their sincere gratitude to the Foundation for the promotion of Zooprophylatics and Zootechnology of Brescia, its President, the Management Committee, the General Secretary, Dr. S. Capretti, Prof. Dr. E. Lodetti and the Secretary Staff for their understanding, encouragement and assistance.

Also we would like to thank Mr. Peter Kettlewell for his invaluable expertise in proof reading the manuscripts and correcting the English language. Finally, our profound thanks to all the Authors who undertook the challenge of preparing a detailed and comprehensive reference work describing an extremely important research topic which previously has received less attention than it deserves!

Fabio Luzi
Malcolm Mitchell
Leonardo Nanni Costa
Veronica Redaelli

PRESENTATION

The Foundation for the promotion of Zooprophylatics and Zootechnology of Brescia, has decided to make an important addition to its series of publications with the inclusion of the Book titled “Thermography: current status and advances in livestock animals and in veterinary medicine”. In this way it hopes to make available a useful, a complete text concerning the Infrared Thermography applied to Animal Production.

The Foundation extends heartfelt gratitude to Editors and Authors of the chapters that have made the publication of this Book possible.

Secretary General
dr. Stefano Capretti

PREFACE

Historically, since the 1980s, the Milan Physics Department has been particularly active in physics applied to environmental and cultural heritage. Non destructive analysis methods have been developed including infrared technologies and thermography in particular. In 2005 cooperation started with the Animal Science Department of the University of Milan. This collaboration is still ongoing and mentioned in this book which contains an exhaustive review of the applications of modern thermography in topics concerning livestock and veterinary science. The book includes contributions from leading research centres in the world and several Italian Universities active in this field.

The initial chapters are devoted to the basic principles of infrared physics and the development of its application, including papers from experts on biomedical and zoological sciences.

It is commendable that a choice of experts in their field have been entrusted with dealing subjects concerning their own specialization.

I believe the book will be of great use to people wishing to deepen the understanding of the applications offered by non-invasive techniques, such as thermography, to zoological studies.

Mario Milazzo

INTRODUCTION

Innovative technologies and biotechnologies contribute greatly to the advancement in animal science. Nowadays one of the most important challenges in this field is to determine reliable measures of animals' overall status. This means being able to assess both the positive and negative responses given by an organism in response to its environment, taking previous experience as well as genetic background into consideration.

To evaluate this response, non invasive indicators may be very useful to obtain reliable data without directly interfering with the organisms, thus avoiding undue stress reactions. Infrared thermography may be a suitable method to reach this goal, it being a non-contact detecting technology. This tool may be successfully used in research on livestock as well as on companion and laboratory animals, but also in research on other animal species.

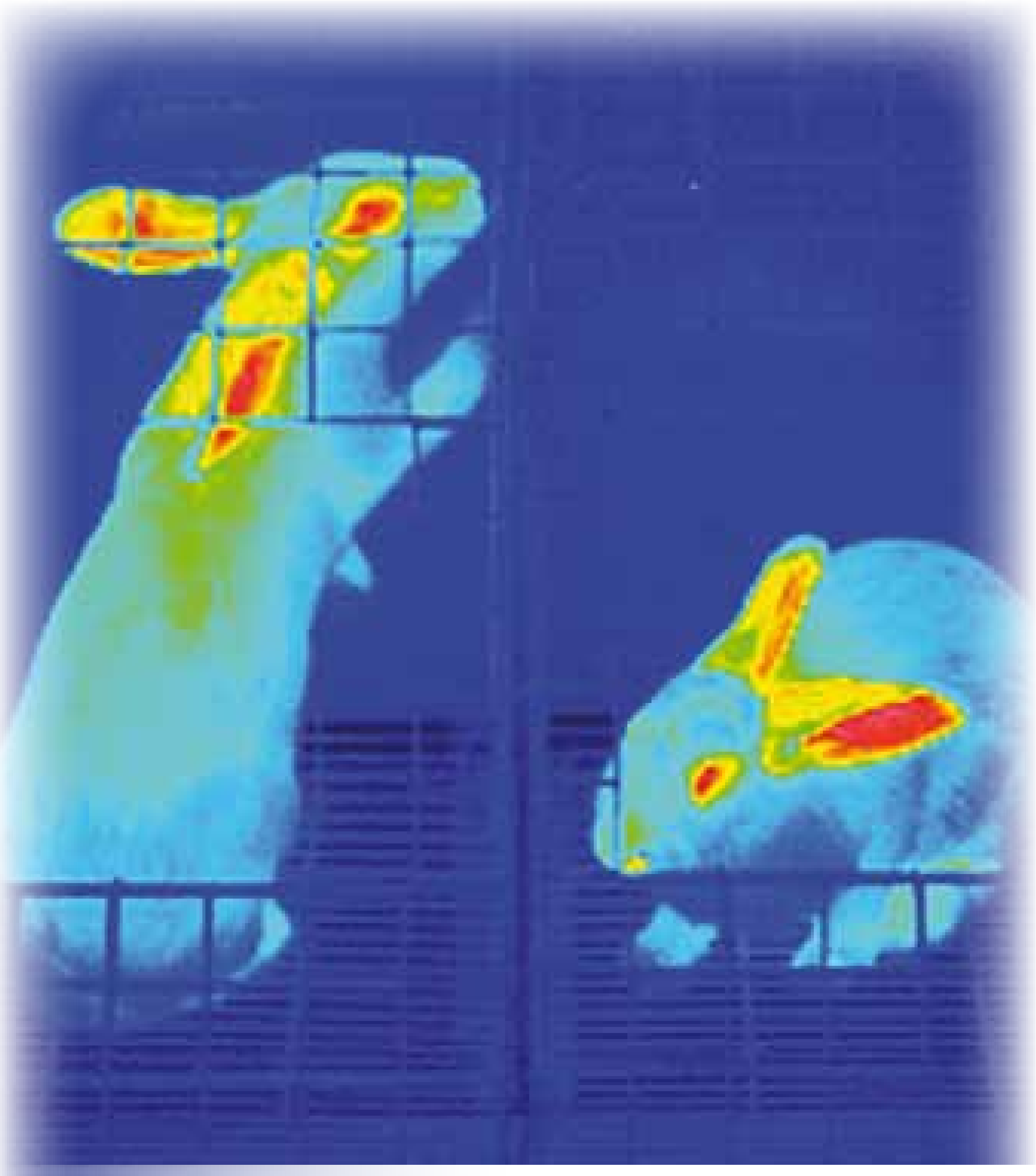
Despite unavoidable difficulties in establishing the most sensible and reliable images and the body areas where temperatures can be evaluated, validating these measurements and then establishing their physiological and/or pathological meaning, infrared thermography has found many biological applications. In veterinary medicine and animal production it has been applied most recently mainly as a potentially diagnostic and preventive tool. It may, for example, be used to detect inflammation or subclinical pathological signs before the disease becomes evident.

In relation to welfare evaluation, thermography measurements may be monitored on unrestrained animals which are free to perform their natural behaviours. The measurements, in conjunction with other scientifically assessed physiological and behavioural variables, may allow the evaluation of the organisms' negative stress reactions. Moreover, data may be recorded remotely both during the daytime and overnight, thus allowing animals to be studied in the field.

This book, starting with the explanation of the scientific and technical fundamentals of thermography, deals with its main applications in livestock and more widely in veterinary medicine. Information concerning the results of this technology obtained from animals' physiology and pathology, and on their reproduction and production, are included, as well as its application to the study of infectious diseases. An interesting field of application is also in animal transport, where thermal images may be very useful in detecting the critical points for animals when travelling long distances at high or low environmental temperatures. Other interesting topics relate to the possibility of applying infrared thermography to laboratory animals and animal care, and to wild and exotic animals.

In conclusion, infrared thermography is an innovative and very promising tool to deepen human knowledge of organisms, and also, from a more practical viewpoint, to improve human-animal interaction and husbandry systems.

Marina Verga



THERMAL IMAGING: HISTORY AND APPLICATIONS

INFRARED HISTORY AND APPLICATIONS

ROBERTO RICCA

INPROTEC IRT, Cinisello Balsamo, Italy

ABSTRACT

The existence of infrared was discovered in 1800 by astronomer Sir Frederick William Herschel curious to the thermal difference between different light colours, he directed sunlight through a glass prism to create a spectrum and then measured the temperature of each colour. He found that the temperatures of the colours increased from the violet to the red part of the spectrum. Practical application occurred only during Second World War. Inspection of electric components has been the most important application from the beginning of introduction in the market of this technology. With the advancing of the IR technologies the applications increased covering, medical, R/D, maintenance, building etc.

Key words

Infrared thermography, Herschel, electric components, maintenance.

THE HISTORY OF INFRARED THERMOGRAPHY

The first name that is mentioned in the context of Infrared radiation is Sir William Hershel who discovered infrared radiation in 1800, and wrote "*investigation of the power of the prismatic colours to heat and illuminate objects ….*".

He stated of his research that: "*It is sometimes of great use in natural philosophy, to doubt of things that are commonly taken for granted: especially as the means of resolving any doubt, when once is it entertained, are often within our reach. We may therefore say, that any experiment which leads to investigate the truth of what was before admitted upon trust, may become of great utility to natural knowledge.*"

In his experiments, Herschel concluded that radiant heat was partly or chiefly made up of invisible light. His work used a prismatic spectrum, the sun light, a white paper and thermometers. Herschel noticed that a beam of radiant heat, emanating from the sun, consists of rays that are differently refracted. The range of their extent, when dispersed by a prism, begins at violet-coloured light, where they are most refracted and have the least efficacy. He traced these calorific rays throughout the whole extent of the prismatic spectrum and found their power increasing towards red-coloured light. After the red light, he described an invisible light "infrared" that generated heat when sunlight was split from a prism.

The first electronic infrared camera was developed by the Germany army and used during the Second World War on tanks for night vision. These cameras could be used only on tanks because they were very large and heavy. At the end of the war the Americans become the leaders in infrared cameras mainly for military applications because it was then possible to see targets in complete darkness.

Between 1950 and 1960 some studies demonstrated that infrared cameras could visualise the early stages of breast cancer, because the tumour cells would draw more blood, creating a hot spot in the thermal image.

A press campaign at the end of the 1950s started to show the applications of this technology in medical and industrial applications. One of the first companies to introduce an IR camera commercially was the Barnes in America, followed few years later by the Swedish company AGA who introduced an infrared camera with the brand name “Thermovision”. This brand name became so famous that today it is still sometime used as a generic name to describe IR cameras.

The first industrial/medical cameras were very heavy. The camera weighed around 20kg with the processor weighing a further 18kg. In addition, to keep the IR detector cooled, liquid nitrogen was required which needed refilling every 2 hours. The presence of liquid nitrogen also limited the degree of tilting of the camera to prevent any liquid nitrogen spilling out.

The first applications were mainly medical for breast cancer diagnosis. Other applications were very limited because the equipment was not portable and required a mains power supply for its operation. To use the equipment for field applications required it to be mounted on a vehicle. In current terms, the price of a camera was around € 350,000.

Other manufactures, such as the Hughes Aircraft Corporation introduced cameras with cryogenic cooling, typically using argon gas to cool the sensor.

The first industry that started to look with interest at thermography was the electric companies because thermography allowed them to visualize hot spots in electric connections of components.

The first steps that increased the uptake of thermography were reductions in the weight and dimensions of the camera and the ability to power the units from batteries. The first portable cameras were introduced into the market in the late 1970s by AGA and though it then allowed field use, the cameras were stil very bulky by modern standards and still using liquid nitrogen for cooling.

The next step in the evolution of thermography was the introduction in the market of thermoelectric cooled sensors, using Peltier cooling. This improved the portability of the devices but the resultant images had lower resolution than those from gas cooled cameras as they operated at 200°K rather than 80°K.

As technology improved, the introduction of micro-coolers allowed the sensor to be kept at 77°K which improved image quality.

In 1997 the first camera with an uncooled focal plane array microbolometer sensor was introduced. This sensor marked a major step forward in the infrared market because it had all the advantages required for manufacturing high volume, low cost IR cameras. The microbolometer sensor works at ambient temperature (300° K), the infrared radiation is captured in the 7.5 -14 micron spectral range where atmospheric transmission is better and solar reflection is reduced. The sensor focal plane array with 320x240 pixels has been a key factor in generating images with relatively good spatial resolution. The current technology uses a microbolometer sensor with a focal plane array starting from 16x16 pixels and now incorporating HD sensors with 1280 x 1024 pixels, a thermal sensitivity of 0.03°C and frame rate until 200Hz.

There are now applications such as active thermography and fast thermography that can be performed with focal plane array sensors using Stirling cooling. With this technology it is possible to reach a frame rate of thousands of images, windowing, setting the integration time from 10 µsec and thermal resolution of 15 mK. These cameras are mainly used for Research & Development and for Lock-in a technique used for NDT applications.

THE THERMOGRAPHIC INSPECTION OF ELECTRICAL INSTALLATIONS

Since the first appearance of thermal cameras in the early 1960s it was understood that one of the main applications of thermography was to identify “*hot spots*” in electrical circuits. The principle of the application is very simple and is based on the Joule effect where, if in an electrical connection the cross section of a conductor decreases then overheating will occur because of the higher density of electric current.

The formula which calculates the amount of heat Q produced by a conductor with resistance R, carrying a current I, in the time interval Δt is:

$$Q = RI^2 \Delta T$$

The heat produced by the Joule effect is therefore directly proportional to the resistance of the conductor and the square of the current passing through it. The electrical resistance of a conductor can now be defined as the ability to convert electrical energy into heat that is passing through it. When an electrical device is required to generate a high heat load the resistance of that device will be increased as much as possible. This happens for example in electrical stoves or in irons. In other cases, however, it is essential that as little energy as possible is dispersed and, although it is not possible to completely eliminate the Joule effect, the heating effect is minimised by using materials with low resistance, such as gold, silver, aluminium or copper. For this reason, the cables that connect electrical devices or those that carry electricity to homes are made of copper.

The main advantages of thermographic inspection of electrical installations are:

- The control is carried out under normal operating conditions.
- The temperature measurement is non-contact.
- Defective components are identified at an early stage when they are not damaged because of interruptions. Often the defective part can be repaired rather than replaced.
- Early detection of defects can improve maintenance and repair programs or the purchase of spare parts.

When the thermographic inspection is undertaken regularly and methodically there will be a considerable reduction of faults.

It is clear that by carrying out an inspection of electrical installations with a thermographic camera only defects that generate a temperature difference are detected. The best condition for thermographic inspection is to have the electrical system in operation with a high load (at least 40%). Some defective components may not be identified because not all anomalies create temperature differences or because at the time of inspection the load is too low.

The factors to be taken into account during the thermographic inspection:

1. Spatial resolution
2. Electrical load
3. Changes in emissivity
4. Reflections
5. Solar Heating
6. Induction
7. Increase in resistance
8. Wind

Each IR camera and lens has a spatial resolution that defines the size of the smallest object whose temperature can be measured at various distances.

The resolution is expressed in mrad and allows, in a simple way, the determination of the size corresponding to a pixel at various distances.

An IR camera with a lens of focal length 21 mm has spatial resolution 1.78 mrad and can measure the various distances an object with the minimum size as indicated in the figure 1 (yellow area).

If an operator wants to measure the temperature of an object smaller than the geometric resolution of the lens, the measured temperature value is incorrect because the dimensions of the target are less than the resolution of the pixel. Taking, for example, the coupling of an insulator of a high-voltage line measured with three different lens.

The spatial or geometric resolution is measured in mrad and the minimum object measurable varies depending upon:
FOV (Field Of View lens)
IFOV (Instantaneous Field Of View lens)
Distance to the object framed
Number of pixels of the sensor

If the dimensions of the object are larger than the area covered by a pixel, then the temperature measurement is correct (left image), if the dimensions of the object are less than the area covered by a pixel, then the temperature measurement is incorrect (right image). Normally, for IR cameras to give the correct temperature reading, there must be at least 2x2 pixels for a correct temperature measurement.

Figure 3 shows an AT line shot taken with a powerful telephoto lens from a distance of 40m. The thermographic image shows that the temperature of the anchoring clamp and the conductor are correct, about 19 °C, equal to the ambient temperature.

In Figure 4 we have a similar photograph of an AT line taken with a medium telephoto lens from a distance of 40m. The thermographic image shows that the temperature of the anchoring clamp and the conductor are correct, about 18.3 °C, while the temperature of the neck is less than in reality since the section of the conductor is less than the spatial resolution of the imager.

In Figure 5 is a photograph of an AT line taken with a normal lens from a distance of 40m. This thermal image shows that the measured temperatures are lower than the ambient temperature as the cross section of the conductor is smaller than the spatial resolution of the camera with the normal lens. A single pixel will indicate the temperature of an area greater than the size of the coupling (measurement area = 28x28 mm compared with the area of the pixel, which is 20x20 mm) The temperature indicated at point A in Figure 5 is 3.5 °C which is obtained from the average between the temperature of the cable (18.3°C) and the temperature of the air (-30°C).

The image of an object on a very cold background, such as the sky, causes an error in the measurement since the average temperature of the pixels is obtained between the ambient temperature and the lower one of the sky.

The choice of lens for each application (size and distance of the object to be measured) is essential to obtain the correct temperature measurement.

INFRARED THERMOGRAPHY FOR MAINTEINANCE

Normal electrical distribution networks are three-phase supplies and for this reason when thermographic inspection is necessary all three phases must be inspected. With a uniform distribution of the load, components such as cables, switches and isolators should have approximately the same temperatures. If one phase proves considerably warmer than the other two, is to be considered defective. It is always advisable to check that the load is distributed evenly over the three phases.

It is important to consider some general rules:

- The middle phase can be slightly warmer than the adjacent ones since there is lower heat exchange in the central area than the side.
- If one phase has a greater load than the other two then it may beslightly warmer, by a few degrees; the central phase is often slightly warmer.
- The camera detects the surface temperature of an object. The operator must be able to analyse its usage and technical capacity, and if there is a difference of a few degrees Celsius on the surface of the component, he must be sure that this mean that there is a much greater temperature inside the component itself.

Figure 7 illustrates a typical example of temperature rise caused by the load.

It is recognised that the infrared energy emitted by a body is proportional to the temperature and the coefficient of emissivity (the Stefan-Boltzmann law is $W = \sigma\varepsilon T4$). An electrical component with a shiny external appearance will have a low coefficient of emissivity, which will reflect the ambient temperature or, if present, any sources of surrounding heat or cold.

In figure 8 we have three aluminium bars. The vertical part is painted red while the horizontal part of its surface is bare metal. The red paint has an emissivity of about $\varepsilon = 0.85$ (points A, B, C,) which results in a temperature of about 46°C. By performing the measurement of temperature on the unpainted part, (points D, E, F,) without varying the coefficient of emissivity temperatures between 28°C and 31°C are measured. These temperatures are not correct, because the coefficient of emissivity is set to red paint, not polished aluminium. The measured temperature is influenced by the reflection of the ambient temperature of approximately 24°C.Inserting the correct emissivity of $\varepsilon = 0.14$, allows the actual temperature of the bar to be measured.

Recognising that that a black body, by definition, does not reflect, if any electrical system has metal parts with shiny surfaces, and thus low emissivity, then care must be taken to avoid reflections. Sources of reflection can be the sun, a lamp, other components or hot surfaces. In this case, the IR camera image shows hot spots which are actually reflections. When working outdoors, the sun is usually the element that creates the reflections. To avoid these problems, it is advisable to make the inspection during overcast days when the sun's rays are less intense. If this is not possible, then it is necessary to view the object from different angles. In this way the points that will be hot from solar reflection will be avoided.

In figure 9 the thermal image represents a heater in an electric cabinet with side panels of polished steel. The cursor A is at the actual temperature of the heater, cursors D, E indicate the ambient temperature in the electric cabinet. The two points, B and C, of the cabinet panel are reflected heater temperatures.

During the day, we generally have a thermal trend of increasing temperature in the morning until the afternoon, after which it cools down into the evening. Bearing in mind that each material has its own value of thermal capacity, in strong sunlight the materials with large differences in thermal capacity, have a different thermal behaviour. Another factor which influences the temperature of a component is its colour and dark coloured components are known to reach higher temperatures than light coloured components. Normally, this causes only small temperature variations which do not affect thermographic inspection.

When performing thermographic inspection of large objects, the thermal map could have shadow areas and sunny areas (figure 11). These will be at different temperatures which are not due to problems but different solar radiation. The problem is very easy to identify because the heat map will match the shadow lines.

REFERENCES

1) Nondestructive Testing Handbook, Third Edition, Editor: Patrick O. Moore (2001).
2) Snell Infrared Canada, Greg McIntosh, P. Eng., Myths and Truths About Wind and Load Correction Factors.
3) Teich, A.C. "Thermography for Power Generation and Distribution", Materials Evaluation Vol. 50, No. 6., Columbus, OH: American Society for Non Destructive Testing June 1992: p. 658, 660, 662.
4) NFPA 70-B, Recommended Practice for Electrical Equipment Maintenance. Quincy, MA: National Fire Prevention Association.

Figure (1). Examples of targets with different geometric resolution.

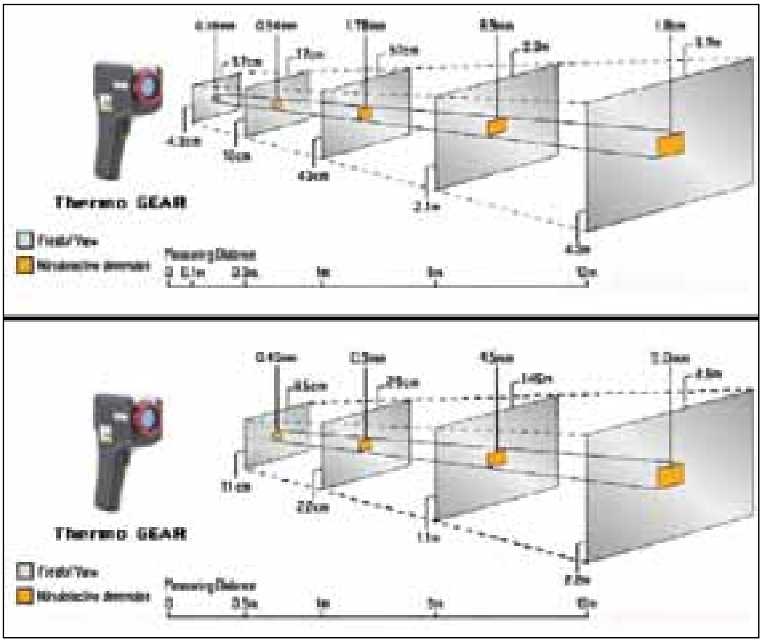


Figure (2). FPA with V x H pixel (Vertical x Horizontal 16x23 pixel in this example).

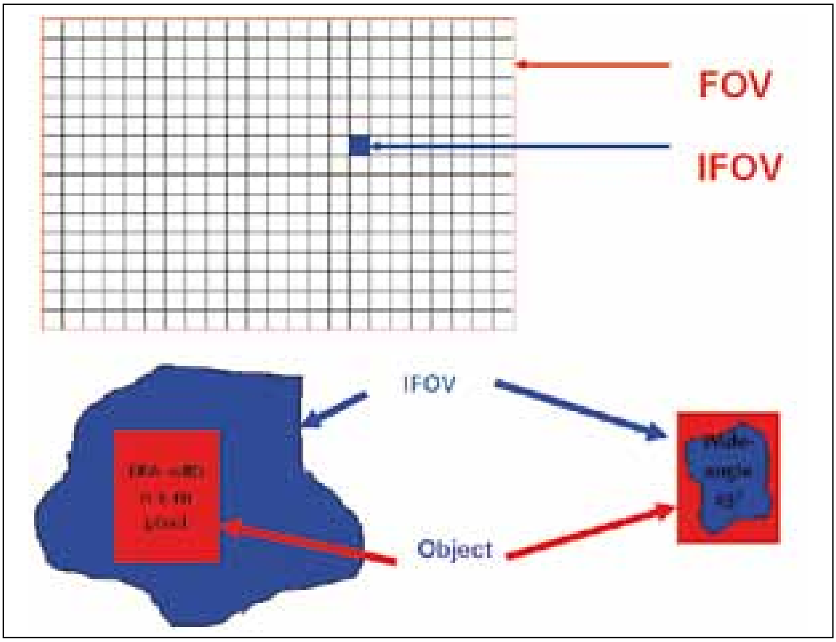


Figure (3). Telescope lens 6x IFOV: 0.22 mrad. Correct temperature measurements.

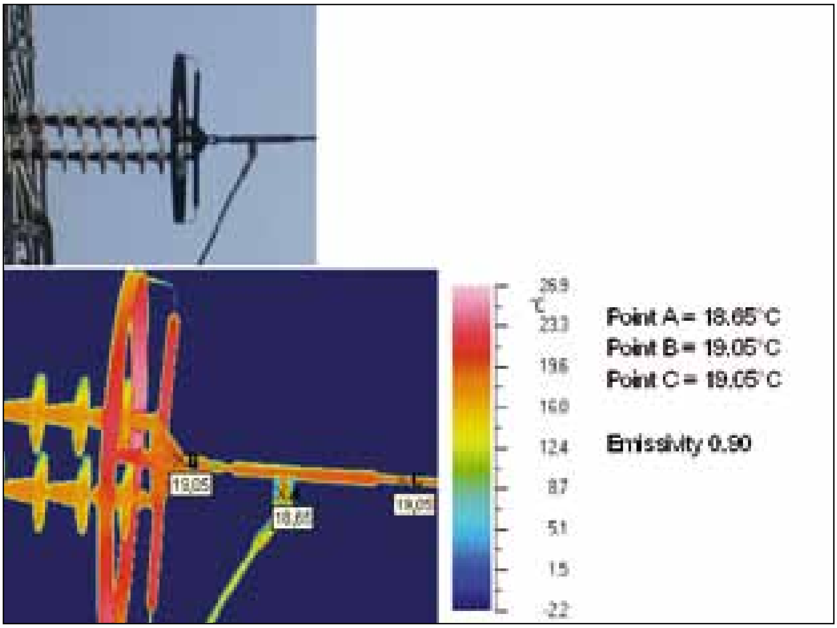


Figure (4). Telescope lens 3x (105 mm.) IFOV: 0.47 mrad. Two measurement correct, one (C) not correct.

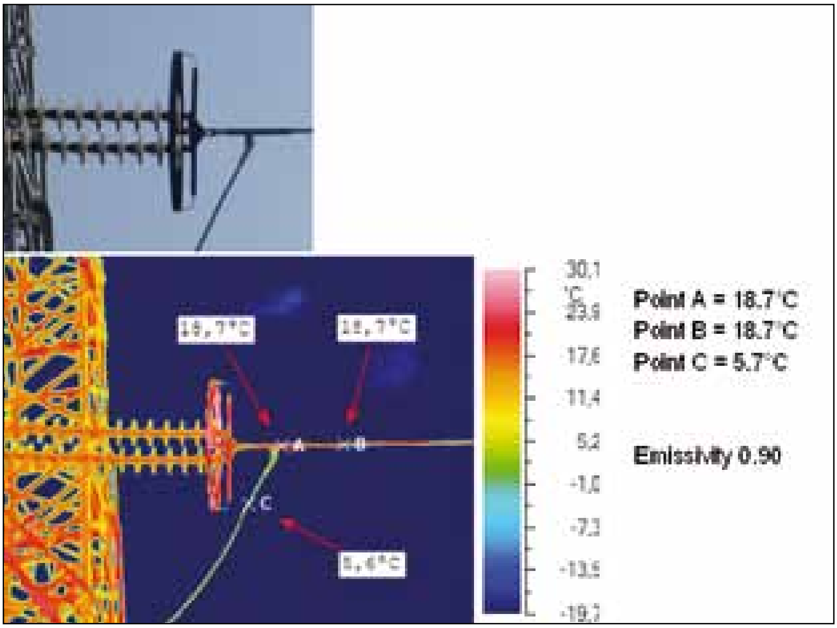


Figure (5). Lens 2x (70 mm.) - IFOV: 0.7 mrad. All points are not correct temperature measurement.

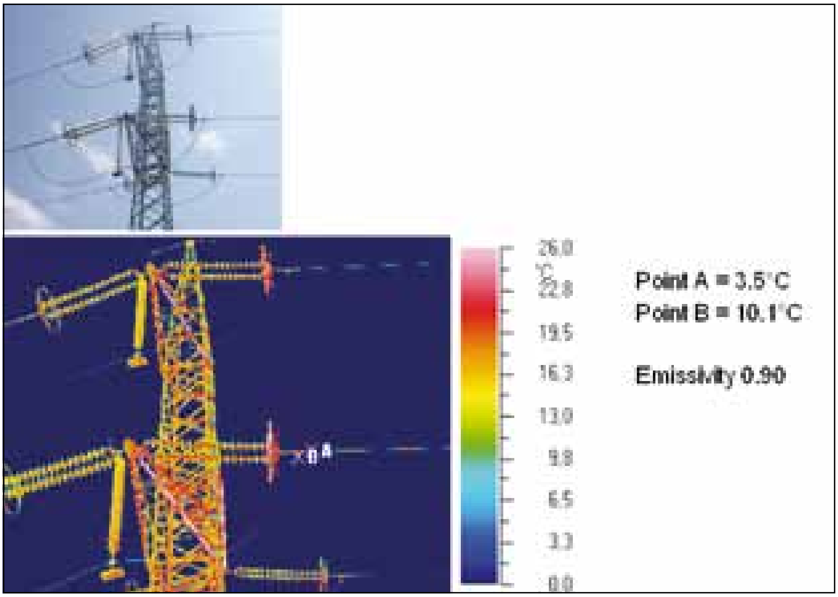


Figure (6). The five thermal images are taken with five different objectives.
The hot spot is not visible with the 90° and 45°. It is visible with the 15° lens but difficult to measure the temperature. To measure the temperature is requested the 6° lens.

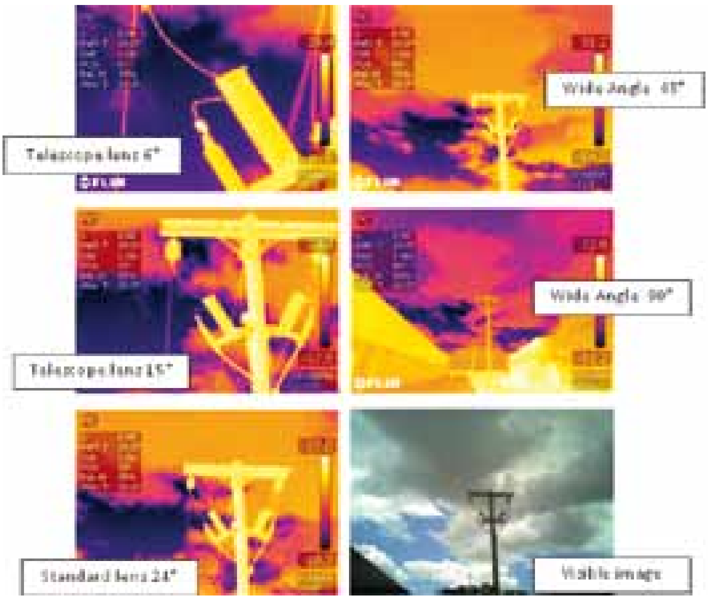


Figure (7). The middle bar is wormer of 3°C for higher load compared external bar.

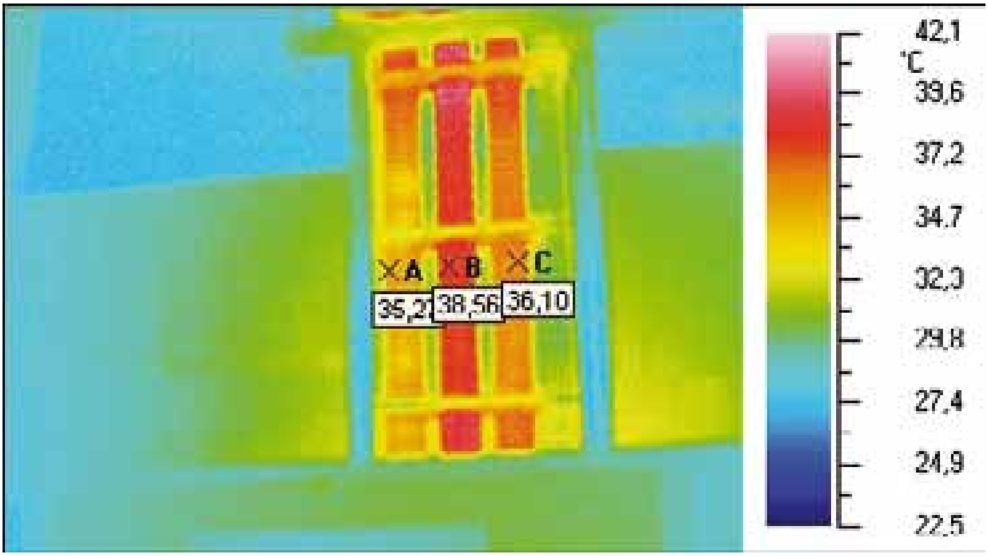


Figure (8). For correct temperature measurement it is important to use the different emissivity, red paint on the bar (E=0.85) and unpainted aluminium (E= 0.14).

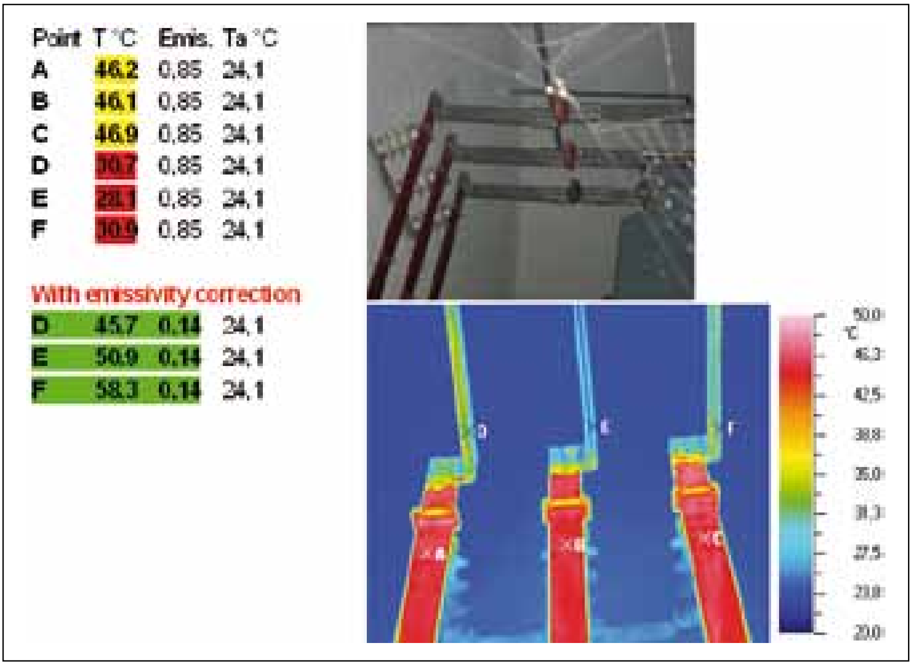


Figure (9). A heater in an electric cabinet with side panels of polished steel.

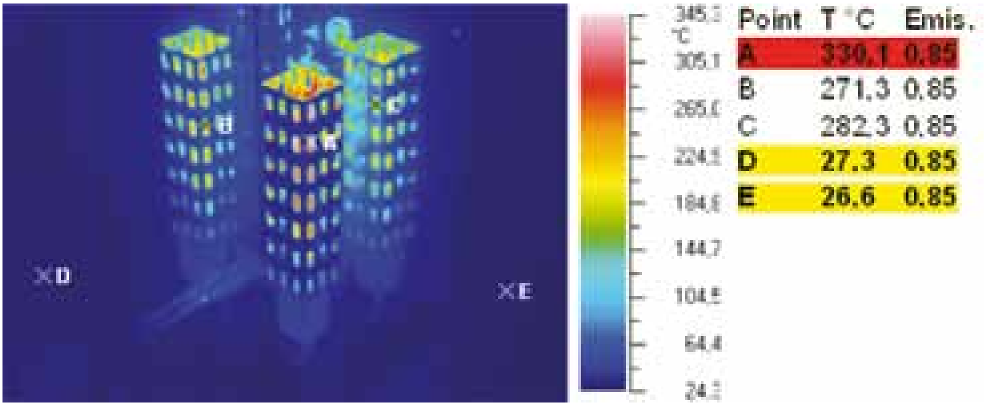


Figure (10). Solar reflection (in red) on the ceramic insulator.

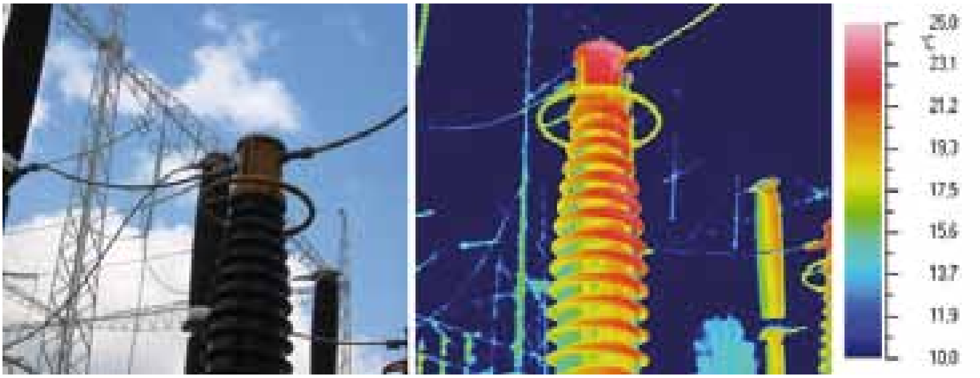
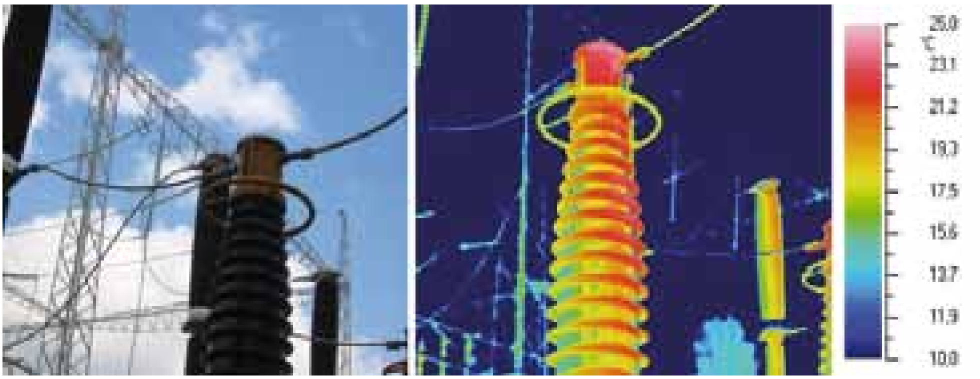


Figure (11). Different temperatures due to different solar radiation.



INFRARED HISTORY AND APPLICATIONS

ELISABETTA ROSINA

Polytechnic of Milan, ABC Department, Italy

ABSTRACT

Infrared Thermography (IRT) is a non-destructive, non-contact method to detect, gather and evaluate information about historic buildings. By mapping the surface temperature and understanding the heat flow, information about a building's materials, their conditions, characteristics, and state of decay can be gathered that may not be evident from visual examination. Furthermore, according to the time of the surfaces heating/cooling process, a map of inner anomalies in the material can be obtained.

Many innovative application, especially the integration with IRT and other NDT, allows to get the target of monitoring the thermal-hygrometrical behaviour of objects and historical buildings in site, and therefore prevent the risk factors for their conservation.

Key words

Restoration, Infrared thermography, Non-destructive testing, historic buildings, moisture, mortar, plaster, stone, detachment, masonry, heat transfer, cracks, porosity, archaeological areas, planned conservation

ASSESSMENT OF HISTORIC BUILDINGS BY INFRARED THERMOGRAPHY

Introduction

Infrared thermography (IRT) is a method to detect, gather and evaluate information about the condition of historic buildings. IRT can provide information about the building elements, their location, shape, material characteristics and state of decay that may not be noticeable from visual examination. This additional information, taking into consideration the environmental conditions, can be obtained by mapping the surface temperature. Furthermore, it is possible to obtain a map of anomalies within the structure of the material from the thermal characteristics of the structure in response to the heating/cooling processes.

The non-contact nature of IRT is advantageous for investigation of delicate or fragile surfaces, where data can be collected from a distance eliminating the need for lifts or scaffolding. Monitoring by IRT can be applied over time, to evaluate effects of treatments, or observe anomalies and changing conditions [1].

Applications to historical buildings

IRT is applied on both modern and ancient buildings, but some differences exist in the testing procedures [2].

The examination of all the available documentation regarding the project and the components of the structure is a necessary prerequisite. However, there may only be a small amount of information available for historical buildings, because sometimes the original drawings and subsequent modifications can be lost with time. IRT is usually the first procedure used in preliminary investigation, surveys and lab tests on materials. Subsequently a direct survey of the materials and the damage is required to know the real state of the surface to investigate.

Other differences in the application of the procedures may be necessary because the walls of ancient buildings are not regular as in modern ones and their thickness, inner structure and number and consistency of layers may vary without this being evident form the external surface. For this reason procedures have to be tailored for each specific case by specially trained personnel. Usually, for ancient buildings only non-destructive testing can be applied and as a consequence, the integration with destructive methods is exceptional and strict limitations are also imposed on the maximum temperature when the active approach is required.

The thermal properties of ancient building materials are not easily found and the range in the values reported by literature is very large. In addition, these properties change with time and environmental conditions. Because of this, quantitative analysis of any defects cannot be obtained, and qualitative evaluation is required. In these cases, the integration with other non destructive tests is essential for verifying the IRT results and cross checking the data.

Investigation for the structural analysis

The objectives of IRT investigations are the detection and evaluation of thermal anomalies resulting from the decay and/or hidden elements of a building. A major application in historic buildings concerns the investigation of inner layers and structural elements which may only be a few centimetres from the surface. It is particularly useful on plaster or stucco and coated masonry construction, where the visual analysis is not enough to distinguish the masonry pattern.

For the *in situ* analysis of plaster/stuccos, procedures are based on the active heating approach by applying a heat flux flowing into the structure.

The surface temperature may give information regarding the inside structure, but to obtain this information it is necessary to apply a heat flux across the structure under investigation. Heat transfer is quicker through the most cohesive materials and/or materials with higher thermal characteristics, so differences of surface temperature due to different thermal properties of elements as timber, bricks, stone and mortar can be visualized as a “foot print” of their shapes projected on the overlapping plaster.

In practice obstacles remain to the extensive application of IRT for the detection of layers that are deep inside the wall, up to 3-5 cm underneath the exterior layer. The difficulty is primarily due to the application of a uniform “thermal load” to the surface to obtain a return signal from the surface which can then be detected by the thermal camera and which could be unequivocally related of the researched defect instead of any surface anomaly.

The artificial application of even heating on wide surfaces can be expensive and time consuming, therefore environment changes in temperature are very useful to obtain the most convenient boundary conditions to detect defects in the buildings’ envelope.

Solar heating is a powerful and even source which can be used to produce a heat flux crossing the structure. The orientation of the building is thus crucial (solar irradiation scarcely hits northern facades, and, depending on the season, also northern eastern facing surfaces may not receive sufficient irradiation for thermographic investigation) as is the shadowing due to nearby buildings, trees, balconies, porches, eaves, etc.

The detection of structural elements underneath the plaster is more successful if the masonry components are cut stone or bricks joined by lime mortar. The pattern is characteristic of the age and the location of the building. The texture of the masonry depends on the size, thickness of the elements and the local building technique at the time of construction. The best results of IRT are achieved in case of stone coated with lime parget [3] because of the higher differences of thermal properties between the stone and the mortar (high thermal conductivity of the stone with regards to the more porous mortar).

IRT investigations in timber require specific procedures because of the thermal properties of the material. Wood provides a good insulation because the material has a low conductivity, therefore only the active investigation approach can be successful. Research developed in the United States shows that wood stud-framed constructions can also be investigated using a proper model of heating and recapturing procedures [4]. However, it has been demonstrated that the qualitative approach can fail if the heating power and duration are not properly calculated and applied. Therefore, a mathematical model of the thermal problem, including the heat exchange between wall construction and the environment, is necessary to obtain reliable thermograms [5]. For that, it is important to monitor the thermal hygrometrical conditions of the ambient environment using probes and data loggers.

Localization of cracks pattern

Another important issue in any structural analysis is the surveying of cracks and their pattern.

The largest cracks are usually visible from a distance, without needing to use scaffolding or ladders. Nevertheless, minor cracks can be difficult to recognize, especially if the surface damage (stains, chromatic alteration, residual decoration, biological attack, incrustation, patina, etc.) masks the presence of cracks.

Thermography is a useful aid because, under transient conditions, it is possible to survey the main cracking and to distinguish if the cracking is only across the coating, across the thickness of the brick, or across the whole thickness of the masonry.

The best survey approach for cracking occurring across a wall is performed when a modulated thermal gradient between the two sides is used. The heat due to mass transfer flows across the cracking and it is possible to survey its shape. In the case of surface cracking the heating of the surface allows a better detection of the edges of the cracking, because of the faster heating due to the delamination of the edge of the cracks. In recent years researchers have developed a suitable technique for the localization of cracks and the definition of their depths [6], that at present has been applied mostly in the laboratory.

Surface defects

Delamination of the finishing

The delamination of the finishing is caused by the creation of an air gap in between the exterior layers of the finishing (including cladding and tiles) and the masonry, or in between the layers of the stucco/plaster itself.

The most frequent cause is water infiltration inside the structure, its evaporation and the crystallization of salts inside the layers of the rendering. The growth of the salts inside the finishing causes the detachment of the exterior layers due to the mechanical pushing of the growing crystals against the pores surface of the material.

The location of delaminated surface is one of the main issues in the maintenance of façades and for the restoration of decorated façades. Traditionally, trained personnel obtain the delamination map by gently knocking the surfaces when scaffolding is set up.

Therefore, the early detection and localization of surface delamination is an important issue for defining the scale of the restoration/maintenance that might be required before starting the repair.

IR thermography allows restorers to have a preliminary map of the defects, without touching the surface. It can be repeated in time, monitoring the develpment of the damage and/or checking the results of the intervention after its application. Nevertheless, by recording thermograams during the heating phase, varying conditions can cause thermal gradients of almost the same temperature span, so the identification of the defects could be questionable. Moreover local non-homogeneities of the surface can cause differences of heat absorption due to the local optical and thermal characteristics of the surface and thermal decay. For example, the chromatic alterations, black crusts, salts, and colors of frescoes and decoration, together with the materials of the tiles and their embedding in the case of ceramic/vitrous finishing, cause localized variations of the temperature pattern. Darker colors absorb more heat than lighter colors and shining coatings reflect the solar irradiation, therefore differences of temperature are not always correlated to defect of adhesion to the substrate.

With the proper environmental conditions, large areas of wall surfaces can be surveyed quickly from ground level by using IRT to map the surface temperature during the heating phase. In a second phase of the assessment, the map identifies the areas where representative sampling should be made to confirm different stucco types and where sounding tests should be made to confirm the areas representative of stucco delamination. These areas can be tested with a rubber hammer, and where it is possible to apply the knocking test without using scaffolding (for example around the windows, at the ground level, etc).

Recent research [7, 8], showed that the most reliable analysis is performed in the transient condition, during the heating of few centimetres of the external masonry layer. Natural or artificial sources of heating successfully generate the convenient thermal gradient for detecting delamination.

Previous studies [9] have demonstrated the advantages of tomographic techniques for obtaining a quantitative approach of IRT on plaster. In such a way the dynamic measures of IRT, in relation to time and the maximum value of thermal contrast, allow the location of the delamination. The procedures can also be applied in the field, often with an uncontrolled boundary condition (for example without a constant irradiating energy) [10].

Integration of IRT, photogrammetry, laser scanning

For the assessment of façades, there is an emerging need for more informative images for gathering the thermal anomalies where the geometry of the object strongly affects the thermal behavior. Such conditions can be due to reflecting materials, non planar surfaces, geometrical patterns of surface decoration and finishing, partial shadows, reflection effects due to objects close to the surface under investigation and places where movement is restricted for data acquisition.

The integration of laser scanner surveying, photogrammetric imagery and IRT allows the generation of 3D multispectral models useful for the localization, visualization, and analysis of anomalies such as damage of finishings, corrosion of the reinforced concrete, heating loss/ lack of insulation, moisture diffusion, etc.

The scientific literature reports research showing the effective use of integrated photogrammetry and IRT in building maintenance and conservation [11, 12]. Nevertheless, one of the well-known major limitations in IRT surveys is directly connected to the reduced angle of view of infrared (IR) sensors adopted in terrestrial applications. In the case of large constructions or when thermal abnormalities are evident only at a larger scale, a single image analysis may not be enough. To overcome this problem, different thermal images can be mapped together on the analyzed building model reducing the distortion of the images.

The integration of IRT, NIR and visual photogrammetry is much more convenient for the localization, detection and measuring of defects and, in the meantime, producing the digital documentation to support the project and maintenance steps (texture 3D models, vector rasters maps of façades, sections, elevations and technical details). The experimental applications of the most advanced research [13] also shows the innovative solution for overcoming the limitations of using targets before the test. At present, the feasibility and costs of the integration are under evaluation.

MOISTURE SURFACE MAPPING

IRT is also used to identify and monitor the diffusion of moisture on or near the surface of masonry. Knowledge of the diffusion of water within the walls is fundamental to the analysis of decay. Current IRT procedures supply a qualitative evaluation of the moisture distribution in the surface and quantitative data of the water content can be obtained by calculating the rate of evaporation flux occurring on the surface under optimal environmental conditions [14].

Using IRT it is possible to calculate the evaporation flux affecting the damp surface because of the variation of surface temperature with time [15].

The visual state of the damage often does not correspond to the current moisture distribution because:

1) the liquid evaporates from the damage surface,
2) the diffusion of water could affects a larger area than the one apparent from visual inspection,
3) the inner layer where evaporation occurs (in case of subflorescences) is often damaged by the process of water movement, but visual analysis reveals this damage only at a short distance.

The new developments in research are combining the advantages and feasibility of the passive approach for locating defects across wide surfaces in real time with the quantitative information provided by active and dynamic applications. The improvement of the modeling phase prior to the investigation assumes a prominent role in the process of planning the procedure, recording the thermograms, data processing and evaluating the results. Despite the reported results [14, 15, 16], at present a limitation remains: the quantitative evaluation of water content using IRT alone cannot measure water distribution deep inside the structure.

The simplest solution for quantifying moisture consists on the integration of IRT with the direct measurements of water provided by other testing, such as weighting tests[1], moisture probes, etc [17].

Recently, many scientific laboratories in Italy have organized a research network (MOdihMa, Moisture detection in historical masonry) focusing on innovative techniques for measuring different parameters related to water content in masonry. The first conference disseminated the initial results using and comparing data that were collected from the experimental laboratory studies and from a study case on a small ancient church, the San Rocco Church, located in northern Italy.

The first objective of this project was to compare the effectiveness of the different techniques and to understand how the quantitative data obtained are directly related to water content. The second objective of the project was to compare the ability of the different techniques to map water as a function of its location and depth within the masonry structure, both on a macro- and micro-scale [18]. At present both the laboratory and in side test are going on for improving the techniques and their application.

APPLICATION ON PLAN OF CONSERVATION: MONITORING

The recent Cultural Heritage Code (2004) [19] defines conservation as a "*planned, long-lasting process, passing previous concepts of "restoration"*". It led to the innovation of the research of testing and studying Cultural Heritage to limit the risk of damage to the existing buildings.

[1] IRT allows to segment the whole surface in zones in which the temperature (and consequently the water content) keeps constant: measuring the water content in just one point of that segment gives information regarding all the area at the same temperature. The result is a drastic reduction of the overall number of measurements necessary and it establishes the possibility to repeat the investigation in different periods in order to observe the dependence of the moisture to the building features, environmental and weather related factors. Sampling and testing procedure is according to UNI 11085 "Beni culturali. Materiali lapidei naturali ed artificiali. Determinazione del contenuto di acqua: Metodo ponderale".

According to the new approach, preventive conservation makes the continuous application of good practices of maintenance necessary, supported by inspection and checking methods and structures to compile the knowledge/assessment data. The plan of conservation also means developing activities to prevent damage, along with the maintenance of buildings [20, 21].

The plan of conservation designs the repair procedures for the building in every step of its life. Before the restoration, this avoids the loss of materials and structure from an unsuitable maintenance program; after the restoration, it has the aim to prevent any possible damage which could occur after the intervention. Diagnostics support both the preliminary assessment and the periodical inspections. The aim of checking and monitoring activities is to control the conservation of the buildings and lead to a faster, better informed interruption of any possible damage.

In recent years the idea of conservation philosophy, preventing the causes of decay from a hazardous environment before the damage becomes apparent, is preferred over restoration after damage has occurred. This innovative approach required the development of monitoring techniques that allow the behaviour of the stone itself and/or the performances of the treated stone to be studied directly on site without taking samples.

Monitoring techniques based on image analysis have a prominent role in the arena of NDT applied to this innovative perspective of the protection of Cultural Heritage, especially in the preliminary phase of the assessment. In fact, the requirements of early detection are in full agreement with the characteristics of such tests since taking images does not require contact with the surface as it is fast and it gives real time results. A single thermogram can be repeated for further comparison with the control over time. Scanning extensive surfaces does not take much time and images of an object, taken in different spectra and thus supplying different information, can be collected in a uniquely informative system. The applications of multispectral analysis in planning conservation are numerous, both if the plan is applied after the restoration, or in developing preventitive measures.

Generally IRT, visual/NIR images are integrated with other low cost monitoring techniques such as monitoring the temperature and Relative Humidity of the air. The goal of early detection meets the requirement of monitoring the condition of the building on site, with robust and reliable procedures. Combining the research approaches has provided innovative laboratory and experimental procedures to produce measurement protocols for on-site diagnostics, thus meeting the requirements of preventive conservation. A brief list of the applications of IRT include the following:

1) Improving the structural analysis to provide an early evaluation of the vulnerability of the structure, by monitoring the change of the patterns of cracks.
2) Detecting thermal imbalance in surfaces and structure which could cause thermal loss and therefore condensation phenomena on the surface.
3) Monitoring the moisture diffusion inside the building structure.
4) Monitoring the state of conservation of surface finishings in the critical zone of the building, for example by measuring the growths of salts or the change of surface porosity on sound and critical pilot areas. New research techniques have been developed for this approach, which are summarized later.

Innovative test for evaluating the surface porosity and hydrophilia in site

Moisture ring and spilling drop

The research takes into account the need to perform non destructive tests on-site for the characterization of materials and conservation products without sampling the materials. At present the main aim is to investigate the porosity of the exterior layers of stone and mortar, and the variation in stone surface performance when it is treated with a hydrophobic product. Further, the new horizon of the research is to study the interaction between the materials and the environment, especially in relation to the outdoor microclimate on-site instead of testing specimens in an indoor laboratory.

To achieve this, the researchers developed two innovative IRT procedures using integrated tests for evaluating the surface thermo-hygrometric properties of stone and mortar specimens. The analysis of the behaviour of the very exterior layer of the material by non-invasive methods, is integrated by traditional standardized techniques and is achieved by the measure of temperatures, obtained by two techniques of passive IRT. The study of the exterior layers of the material is crucial in evaluating the decay advance due to the water exchange and the changes in the surface hydrophilicity due to the application of restoration products (consolidants and water-repellants). Current standards [22-23] and the most advanced testing procedures in European Union [24] indicate the measurements of porosity, the absorption by capillarity and the drying index needed for a complete material characterization. Their measurements are obtained with weighing tests along with the average water absorption by the whole thickness of the specimens.

Both of the innovative methods analyse the evaporation of a small quantity of water applied on the surface. The first method, Spilling Drop (SP), uses the application of water without any pressure and consists in free spilling of a drop of known volume on the plane surface of the specimen. In the second method, Moisture Ring (MR), the application of water is performed by means of a dampened sponge applied with controlled pressure [25].

The results of laboratory testing to date show a clear correlation between porosity, evaporation flux (drying index), absorption by capillarity and water spreading phenomena (both in liquid and vapour phase) inside the microstructure of the external layer of the material measured as cooled areas on the sample surface.

Spilling drops test (SD)

This test allows the measurement of the surface characteristics of absorption and diffusion of liquid water by recording a thermal video sequence, at optimal ambient condition (air temperature = 23.5°±1° C, RH = 30±4 %). Images are taken at a rate of 1 Hz, for a 10 minute sequence. During the filming, one drop of distilled water (0.03 ml) is spilled on the sample surface. The sequence of the images show the water spreading on the surface according to the porosity of the surface. In this case the parameter used to determine the porosity was the area of the damped surface cooled by the evaporation of spreading water.

Moisture ring test

The contact sponge test was monitored by IRT. A continuous video captured 10 minutes of evaporation starting at the end of the sponge application. The procedure allows the observation of the differences in water spreading on the different stone surfaces. In both tests, evaporation of water causes cooling of the wet areas, and due to the cooling the thermal contrast between wet and dry areas is higher when the evaporation flux is higher [26] due to the differences of porosity. At present the researchers are studying the validity of the tests at critical environmental conditions.

APPLICATION ON ARCHAEOLEGICAL AREAS

The role of nondestructive testing (NDT) in the protection of archaeological sites, is very important. Microclimate, surface temperature and solar irradiation, as well as wind direction and speed, moisture diffusion and rising damp, can cause damage to archaeological remains especially because of diurnal variations.

To understand better the influence of these factors on the damage to archaeological remains and to learn how best to prevent damage, the researchers performed an outdoor monitoring of all these factors over a five year period on some archaeological areas in Sardinia [27] .

The results of the monitoring helped to define the requirements of innovative shelters that should prevent the risk of condensation (occurring overnight and in cold weather), the increase of surface temperature (due to the solar irradiation, especially on the exterior sides during the summer sunsets and dawns, when the inclined sun beams hit the ruins despite of the installation of shelters). In addition, several technical aspects were analyzed highlighting how traditional covering solutions can often contribute to damage instead of offering protection. To overcome the limits of typical protective building systems, the project needed to combine the requirements for preservation and identify new approaches of design. Some of the requirements for preservation included compatibility and reversibility of new materials, as well as protection from environmental aggression. The new requirements of the project include flexibility, feasibility, low maintenance and easy disassembly to allow reuse of the shelter in different locations and/or seasons.

This research was based on a multidisciplinary approach that included the integration of documentation, collection of experimental data regarding the environment and its effects on the findings, the design of a mock in-situ triage of the materials and structural performance and its influence on the findings and laboratory tests on the material performance before and after 15-30 months of natural aging, including the effects of pollution and weathering.

The experimental measurements supported the design process. The mock-up of the two multilayer textile shelters went through on-site testing in order to measure the real performance for reflection of solar irradiation. The stability performance after aging was also examined with the use of spectrophotometrical and thermal analysis of the samples before and after prolonged exposure to polluted air.

CONCLUSION

The successful application of IRT to historic buildings and archaeological areas requires an understanding of the materials under investigation. Ancient materials often have untested thermal properties. Without laboratory tests of these materials to fully understand their thermal characteristics, the investigation will be limited to a qualitative evaluation and even that can sometimes fail.

It is important to select the most significant areas on which the investigation has to be performed and the target on the basis of the available preliminary information and documentation.

Determination of the correct time and duration to perform testing is critical because buildings are subjected to slow and varying boundary conditions and different heat fluxes may interact causing detrimental or additive effects which significantly filter the thermal signal. By contrast, depending on the dimensions, mass, materials and location, the archaeological remains may present a fast variation of the surface temperature due to their complete exposure to the surrounding environment. Therefore it is essential to follow a well tested and robust procedure for applying on-site tests in order to control the effects of the boundary conditions.

IRT can be a reliable investigative tool for the detection of subsurface materials and anomalies. It is entirely non-destructive and non-contact with the ability to cover a large area in a relatively short amount of time given the correct boundary conditions. The increase in performance of the new generation of devices makes it possible to obtain detailed information with a reduced cost and in particular the improvement of the imaging and data processing allows better information to be obtained at the same costs.

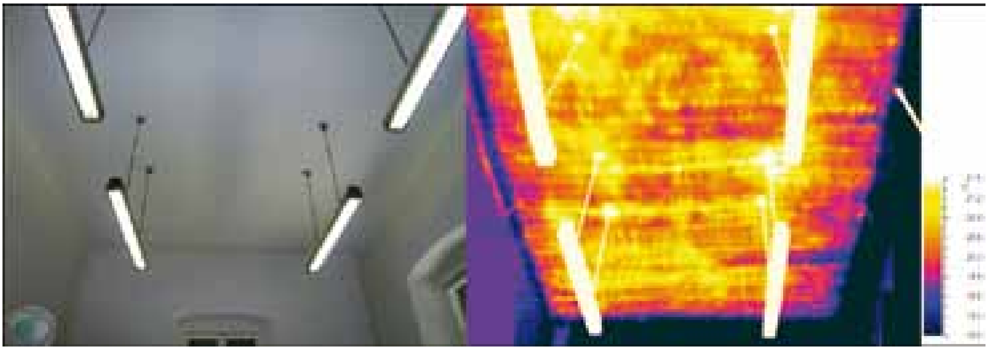
However, it is important to understand that IRT gives only measures of radiated surface temperature which is influenced by emissivity and any reflected radiation. The interpretation of these temperature maps relies on the correct careful and thorough evaluation of IR data in conjunction with the correct evaluation of thermal parameters and a full understanding of historic building materials and their construction and the entire thermal environment.

BIBLIOGRAPHY

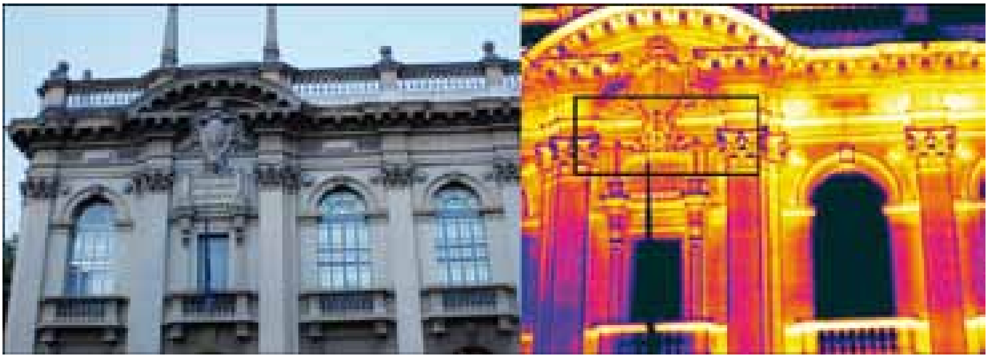
1) Rosina, E. and Spodek J., "Using Infrared Thermography to Detect Moisture in Historic Masonry." *APT Bulletin: Journal of Preservation Technology*, Vol. XXXIV, No. 1 (2003): p. 11-16.
2) E. Rosina, E. Grinzato, Infrared and Thermal Testing for Conservation of Historic Buildings, in Materials Evaluation, ASNT Journal, volume 59/n 8, Agosto 2001, ASNT Columbus (OH) USA, pp. 942-954
3) Grimoldi A., Grinzato E., Rosina E., Vavilov V., Di Flumeri E., Roche G. and Papetti V. "Capturing The Building History by Quantitative IR Thermography." paper presented at the *3rd International Congress on Science And Technology For The Safeguard of Cultural Heritage in The Mediterranean Basin*, Alcalá de Henares, Spain, July 2001.
4) Rosina E. and Robison E. "The Applying of Infrared Thermography on Historic Wood Framed Buildings in North America." *APT Bulletin, the Journal of Preservation Technology*. Vol.XXXIII, No. 3 (2002): pp. 37-44.
5) Rosina E., Grinzato E. and Robison E. "Mapping Masonry Bonding by Quantitative IR Thermography." in *Thermosense XXIV*, SPIE Proceedings, Vol. 4710. Bellingham, WA: International Society for Optical Engineering (2002): pp. 253-264.
6) V. Vavilov S. Marinetti E. Grinzato, Infrared thermographic nondestructive testing of frescos: thermal modeling and image processing of three dimensional heat diffusion phenomena, Materials Evaluation, Volume 60 N. 3, pp.452-460, March 2002.
7) V. Redaelli, S. Caglio, M. Gargano, N. Ludwig, E. Rosina, The surfaces of contemporary architecture: characterization of clinker by IRT, proceedings of AITA, L'Aquila 2011, pp. 1-10.
8) N. Ludwig, E. Rosina, S. Caglio, M. Gargano, V. Redaelli, Heat Diffusion in Klinker Facade: The Study Case of a Giò Ponti Building, Advances in Optical Technologies, on line journal, Volume 2012 (2012), Article ID 738943, 6 pages, Hidawi ed.
9) V. Vavilov, P.G. Bison, C. Bressan, E.Grinzato, S Marinetti, Some new ideas in dynamic thermal thermal tomography, QIRT 1992.
10) P. Bison, M. Dezzi Bardeschi, E. Grinzato, T. Kauppinen, E. Rosina, G.Tucci, Infrared Thermal Scanning as a Methodology for Survey of Facades Palazzo della Ragione, Milan, Italy Thermosense XVIII-An International Conference in thermal sensing and imaging, diagnostic applications, Orlando USA. 1996.
11) Ribarić, S., Marčetić, D. and Vedrina, D.S., "A knowledge-based system for the non-destructive diagnostics of façade isolation using the information fusion of visual and IR images", Expert Syst Appl 36(2):3812-23, (2009).
12) Palaia L., Sánchez R., López V., Gil L., Monfort J., Tormo S., Navarro P., Álvarez M. Á., Procedure for NDT and Traditional Methods of Ancient Building Diagnosis, by Using Thermograph, Digital Images and other instruments data Analysis, Proceedings of the 17th World Conference on Nondestructive Testing, 25-28 Oct 2008, Shanghai, China.
13) M. Alba , L. Barazzetti, M. Scaioni , E. Rosina, M. Previtali, Mapping Infrared Data on Terrestrial Laser Scanning 3D Models of Buildings, Remote Sensing, 2011, 3(9), pp.1847-1870.

14) Rosina E. and Ludwig N. “Optimal thermographic procedures for moisture analysis in buildings materials.” *Diagnostic Imaging Technologies and Industrial Applications Conference”*. SPIE proceedings, vol. 3827, Bellingham, WA: International Society for Optical Engineering (1999) pp. 22-33.
15) N. Ludwig, V.Redaelli, E.Rosina e F.Augelli: “Moisture detection in wood and plaster by IR thermography”, Infrared Physics & Tecnologies 46 (2004), pp. 161-166, Elsevier.
16) Tavukçuoğlu A. and Grinzato E. “Determination of Critical Moisture Content in Porous Materials by Infrared Thermography.” Paper given at QIRT [Quantitative Infrared Thermography] Padova, Italy (2006).
17) Cruciani Fabozzi G., Rosina E. and Valentini M. “Thermography and gravimetric tests in moisture analysis.” *XX Congresso Nazionale AICAT - GICAT, Metodi chimici, fisici e biologici per la salvaguardia dei beni culturali [Chemical, Physical and Biological Methods for Safeguarding Cultural Assets*]. Published proceedings. Rome, Italy (1998): pp. 29-47.
18) Materials Evaluation n 1, vol 69, ASNT ed, Columbus, OH-USA, 2011.
19) Legislative Decree n 42 of 22 January 2004, Code of the Cultural and Landscape Heritage, http://www.unesco.org/culture/natlaws/media/pdf/italy/it_cult_landscapeheritge2004_engtof.pdf
20) S. Della Torre, E. Rosina, Rapid techniques for monitoring historic fabric in preservation plan, SMW08, International Workshop on In situ monitoring of monumental surface, Firenze, ottobre 2008.
21) S. Della Torre, E. Rosina, C. Faliva, M. Catalano, *Sperimentazione di tecniche analitiche integrate per la diagnostica finalizzata alla conservazione preventiva*, in Atti del IV Congresso Nazionale IGIIC, Lo stato dell’Arte, Siena 2006, Nardini Ed. Firenze 2006, pp. 313-320.
22) UNI 10859 (Materiali Lapidei Naturali ed Artificiali. Determinazione dell’Assorbimento d’Acqua per capillarità) 2000 - Milan., Italy.
23) Misura dell’indice di asciugamento (Drying Index) Normal 29/88, NORMAL F metodologie fisiche meccaniche.
24) EN 1015-18 (2004: Methods of test for mortar for masonry: water absorption coefficient due to capillary action of hardened mortar).
25) C. Pardini, P. Tiano, Valutazione in situ dei trattamenti protettivi per il materiale lapideo. Proposta di una nuova semplice metodologia, in Arkos 4/03, Nardini ed. Firenze 2003 (Italy),32-38.
26) E. Rosina, N. Ludwig, S. Della Torre, S. D’Ascola, C. Sotgia, P. Cornale, Thermal and hygroscopic characteristics of restored plasters with different surface textures, in Materials evaluation. - ISSN 0025-5327. - 66:12(2008 Dec), pp. 1271-1278.
27) E. Rosina, A. Zanelli, P. Beccarelli, M. Gargano, E. Romoli, New Procedures and Materials for Improving Protection of Archaeological Areas, Materials Evaluation, n 81, vol 69, ASNT ed, Columbus, OH-USA, 2011, pp. 979-989.

Figures 1 and 2. The ceiling of a ground floor room at Villa Carrega, Genua and the corresponding composite thermograms. Investigation in of the thermal IR and shows localized detachment of the plaster (white spots) and the pattern of the ceiling timbers providing the structure of the plaster.



Figures 3 and 4. The western façade of the Rector building at Polytechnic of Milan. The InfraRed mosaic shows many thermal anomalies due to the damage of the decorative cement, although many thermal gradient are due to the prominent decoration (in square 1) that protect the surface from the direct solar irradiation. Temperatures of the surface range between 30 and 33°C.



Figures 5 and 6. Monastero del Lavello in Calolziocorte, western elevation: composite thermograms show the infilled opening (rectangles 1), the stone quoins of the corner (square 3), different thickness of the stucco layers. On the arched portal there are some sealed cracks (2).

Figures 7 and 8. The Palazzo Marchesale in Melpignano (Lecce). The IR Thermogram magnifies the length and size of the smallest cracks (in the white squares).

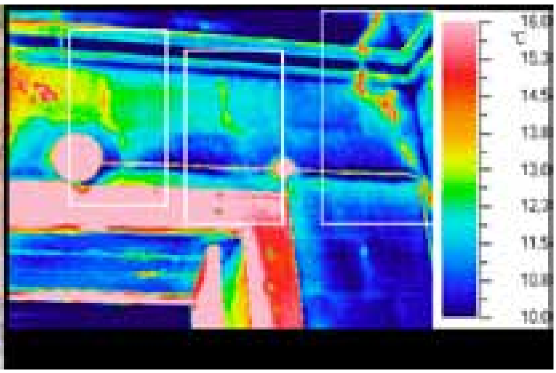
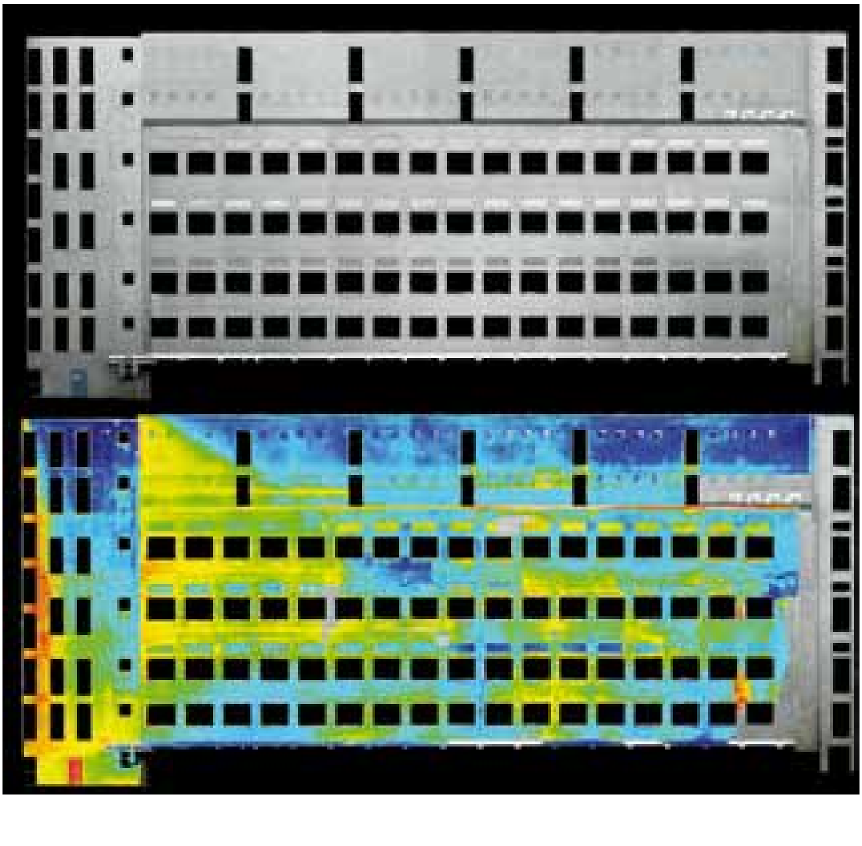
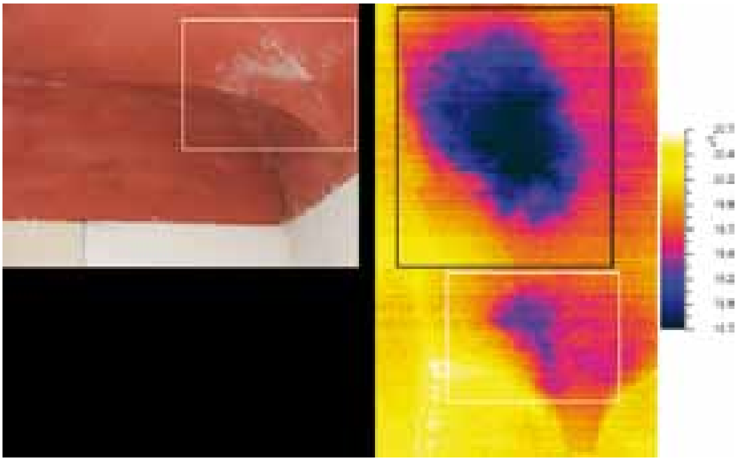


Figure 9 and 10. In a ground floor room at the Villa Carrega, Genua, the mosaic of thermograms shows the plaster delamination extent of damage is tappears wider at the IR spectral band than the visual one. The delamination of plaster (warmer areas) is due to the huge water infiltration from the top of the building.

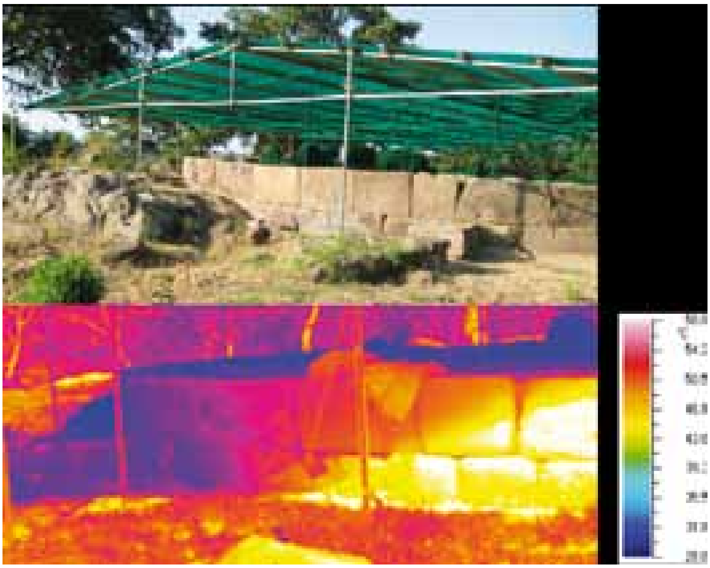
Figure 11 and 12: southern facade of the Building "La Nave" by Gio Ponti, Campus Leonardo, Polytechnic of Milan. Photogrammetry of the composites of the visual and InfraRed images.



Figures 13 and 14. The Moneta Palace, Inzago (MI). The IRT composite shows recent water spreading in the vault (the black square in Figure 14) before the damage appears. The efflorescing salts (in the white square of Figure 13) indicate a past water infiltration which is almost dry (in the white square of Figure 14).



Figures 15 and 16. The western side of Nuraghe's Shrine, Sorradile archaeological area. The InfraRed image recaptures the stone quoins at noon in July when the solar irradiation hit the side of the temple remains. The difference of surface temperature between the shadowed top and the irradiated side is about 30°C. Differential expansion of the material causes stress in the quoins, and generates micro-cracks, blistering, and splitting of the exterior layers of the stone (trachite). At dawn and sunset the thermal gradient is lower, but the surfaces directly hit by the solar irradiation are wider, because of the inclination of the sun beams.



INFRARED HISTORY AND APPLICATIONS

NICOLA LUDWIG

Physics Department, State University of Milano

ABSTRACT

Thermal imaging allows the visualization of differences in surface temperature by detecting infrared radiation emitted by anybody in two spectral atmosferic windows at 3-5 μm and (8-14 μm). Trought specific algorithm these radiation data can be transformed into images in scalar grey levels. Gray levels images finally can be converted in false color scale in which a color reference scale is used to put in evidence thermal anomalies. In the field of biological applications infrared thermography (IRT) is used for visualizing stress-induced changes in leaf transpiration. Changes in leaves mainly results from alterations of transpiration in response to particular stresses and can be used to detect rate stresses as well local water content. About applications on human, in thermographic analysis, the most important is that skin is an interface, more or less conductive, between an inner warm core and an outer colder environment. Thermal imaging is devoted to reveal the presence of heat dissipation processes under this surface.

Key words

Infrared, thermography, non-destructive evaluation, heat transfer model, skin temperature, stress, thermoregulation.

THERMAL IMAGING IN BIOLOGICAL APPLICATIONS

Thermal imaging allows the visualization of differences in surface temperature by detecting infrared radiation emitted by any body in two spectral windows at 3-5 μm and 8-14 μm. Using specific algorithms these radiation data can be transformed into images in scalar grey levels. Gray level images can be converted into a false color scale in which a color reference scale is used to identify thermal anomalies. In the field of biological applications, infrared thermography is a straightforward choice for visualizing stress-induced changes in leaf transpiration under given controlled environmental conditions (Jones, 1999). Infra Red Thermography (IRT) is recognised for its application to sense crop vitality as the temperature of plant leaves is determined by environmental factors and cooling due to evaporation from stomata (Milazzo et al., 1994). Changes in plants mainly result from alterations of transpiration in response to particular stresses and can be used to detect evapotranspiration rate or water loss stresses as well local leaf water content (Figure 1).

Modifications in the water content of a plant caused by adverse conditions lead to changes in leaf transpiration as a result of active regulation of the stomatal aperture. The associated changes in leaf cooling can be monitored instantly by thermography (Ludwig et al., 2010). The basic principle of this technique is the relationship between leaf temperature and leaf transpiration. Several authors demonstrated that, for porous materials, at room temperature, there is a linear relationship between the cooling due to the evaporation recorded by an Infra Red (IR) detector and the flux rate (Bajons et al., 2005). Therefore, temperature variations of the leaf surface depend strongly on the plant water status, which, in turn, is a function of the stomatal conductance.

Studies also show that the thermal effect provides an indication of the final extension of necrosis on both plants and canopy level (Leinonen et al., 2004). In a study on tobacco and *Arabidopsis* with cell death mutants that form lesions resembling a pathogen-induced hypersensitive response, a cooling effect was recorded due to the evaporation flux from leaking cells before any visual damage can be visually detected (Chaerle et al., 2002).

IRT testing can be divided into two branches: active thermography permits non-destructive determination of leaf water content in relation to the change of heat capacity of tissue, whereas passive thermography measurements can be used to estimate stress related changes in the amount of water evaporated in term of vapour flux rate kg $m^{-2}s^{-1}$]. Although passive thermography can be used to locate emerging disease outbreaks, as shown by its current use in aerial remote sensing at the field scale, it does not characterize the stressor. When developing thermography at field scale, its sensitivity to changing environmental conditions (solar irradiation, wind, clouds etc.) should be taken into account, otherwise the influence of environmental changes would be superimposed on the thermal signature of the stress under study.

Under controlled conditions, thermal imaging has enabled the isolation of stomatal mutants through screening assays (Morandini et al., 1996, Mustilli et al., 2002) as well as the presymptomatic visualization of a number of biotic plant stresses (Boccara et al. 2001). The hypersensitive response of resistant tobacco to Tobacco Mosaic Virus (TMV) infection is a well-characterized model system. Using thermography a presymptomatic temperature increase at sites with TMV infection was found, which overlapped with localized salicylic acid accumulation and it was thus conluded that the local temperature is affected by stomatal closure induced by the pre-necrotic accumulation of resistance-associated compounds (Chaerle et al. 2002). The same researchers also showed high-contrast thermal detection of pre-necrotic symptoms in tobacco and *Arabidopsis.* Several authors hypothesized that any local plant cell death phenomenon would be detectable by thermal imaging at an early stage. Skaracis and Biancardi (2000) found that *Cercospora* leaf thermal anomalies are connected to a necrotrophic fungal infection of sugar beet, characterized by the formation of dark circular necrotic spots. This symptomatology thus provided a testing opportunity for the wider applicability of early thermal disease detection. Plant resistance in general, with or without the manifestation of hypersensitive response, inevitably has repercussions on general metabolism and plant physiology. Different effects on photosynthesis and transpiration can possibly be revealed by chlorophyll fluorescence imaging and thermography respectively depending on the metabolic reactions affected by plant–pathogen interactions (Chaerle et al., 2004). Finally Jones et al (2002) studied the same effects of conductance in crops of grapefruit plants.

Infrared thermography can be a useful approach for both proximal and remote sensing of plant biotic stresses, for irrigation scheduling in arid environments, and for screening the stomatal functionality in different lines of a crop of interest (Hellebrand e al., 2002). Combined procedures and technologies can improve the plant water-use efficiency. In this context, under drought conditions, the use of antitranspirants may improve the water-use efficiency often assumed to express the irrigation system performance and also defined as the ratio between the crop biomass and the amount of water consumed by the crop itself, including rainfall, irrigation water and plant transpiration. Antitranspirants are compounds applied to foliage in order to limit water loss (Pereira et al., 2002).

Energetic balance of leaf

Thermal imaging by infrared thermography represents a suitable system for studying the energy balance of the surface of leaves especially for the estimation of evapotranspiration rates. They include both film-forming and stomatal-closing compounds, able to increase the leaf resistance to water vapor loss and improve the plant water-use efficiency. In recent work, Ludwig et al. (2010) have studied the antitranspirant activity of chitosan (CHT) in bean plants. CHT is a natural, not toxic and low cost compound obtained from crustacean, insect and fungal chitin deacetylation, the second most abundant biopolymer after cellulose. Problems concerning the correlation between thermographic measurements and physical variables, directly related to thermodynamic processes involved in leaf transpiration, have already been studied. The fundamental result was that thermography was able to measure differences of tens of bar in water potential (W), and to record them as a difference of temperature of some fraction of degree.

The leaf transpiration can be obtained as the rate of evaporative flux in a non-invasive and remote sensing way, through the measurement of leaf temperature. The evaporation phenomenon has a strong influence in the energetic exchange processes of the leaf and, therefore, in its equilibrium temperature in respect to the environment. The main advantages of the thermographic technique applied to plant inspection are the possibility of a wide use over crops, the quickness and the non-destructivity. When all environmental variables (irradiation, air temperature, relative humidity and ventilation) are fixed, the leaf temperature does not change and the non-contact measurement of temperature can give the amount of energy exchanged between the environment and the plant (Nobel, 1983). The regulation of water vapor rate lost during transpiration is very important in plant thermoregulation, because of the high value of the heat of vaporization (2.25 x 10^6 J/kg). The sum of the different energies exchanged in the equilibrium condition, i.e. when leaf reaches a stable equilibrium temperature, must be equal to zero:

$$Q_R + Q_C + Q_T + Q_W = 0 \quad (1)$$

where: Q_R sums all the terms of incoming and out going irradiation; Q_C is the term due to the convection exchange: Q_T is the energy exchanged for mass transport; Q_W is the heat of vaporization. Substituting all the terms in (1) we can write it as:

$$\alpha\varepsilon_{env}\sigma T_{env}^4 - \varepsilon\sigma T^4 - h(T - T_{air}) + c_p\Phi_w(T_{air} - T) - \lambda\Phi_v = 0 \qquad (2)$$

Where: α=absorbance; ε_{env}=effective coefficient of environmental emissivity; ε= leaf emissivity; σ= Stefan-Boltzmann constant; Φ_v= vaporization rate from the leaf; Φ_w= water flux going into the leaf; λ= heat of vaporization; T=leaf temperature; T_{env}=environmental temperature; h= Newton's coefficient of convection exchange; c_p= constant pressure specific heat of leaf.

This expression for the energetic balance of a leaf can be connected to the stomatal conductance throught Fick's law of diffusion:

$$\Phi^* = -D\frac{\partial C}{\partial x} \quad (3)$$

Whereevaporation flux Φ^* is expressed in [mol $m^{-2}s^{-1}$], C is the molar concentration [mol m^{-3}] and D is the diffusion coefficient [m^2s^{-1}]. The importance of measurement by thermography of --m is the ability of obtaining an evaluation of stomatal conductance avoiding any contact with leaves.

In the condition of equilibrium among all the components of equation (2) the solution is given by the equilibrium temperature, T_α that depends on all the physical constants. This temperature can be measured with a thermographic system, but, in the measurement it is impossible to distinguish the zones where the evaporation actually takes place (stomatal opening), from those where there is no evaporation (cuticular surface of the leaf). In this model, the flux rate can be obtained by the following expression:

$$\Phi_v = \frac{\alpha\varepsilon_{env}\sigma T_{env}^4 - \varepsilon\sigma T^4 - h(T_\alpha - T_{air}) + c_p \Phi_w (T_{air} - T_\alpha)}{\lambda} \quad (4)$$

In non stress water conditions, since the speed of capillary diffusion by suction is higher than the gaseous diffusion, the amount of mass lost by evaporation can be considered equal to the mass of fluid coming from the plant's root. The other components relating to transpiration are the fluid mass transport from roots to leaves and the associated energy transfer. However a simple assessment shows that in a standard conditions, such as temperature difference between shaft and leaves less than 10°C and environmental relative humidity (RH) between 30 and 60%, the energy associated to a given mass of fluid transported within the plant is about two orders of magnitude lower that the energy needed to let the same mass evaporate. The energy required to evaporate a gram of distilled water is about 2200 J while the energy for the transfer of the same mass within the vascular system, considering a ΔT of 5°C, is of the order of only 20 J.

So we have that $\Phi_v = \Phi_w$, and then:

$$\Phi = \frac{\alpha\varepsilon_{env}\sigma T_{env}^4 - \varepsilon\sigma T^4 - h(T_\alpha - T_{air})}{\lambda - c_p(T_{air} - T_\alpha)} \quad (5)$$

The relation (5) between evaporation flux and equilibrium temperature T_α has been experimentally verified by means of both direct measurement of temperature and evaporation flux obtained by weighting (Figure 2). Measurements in laboratory conditions allow the estimation of the environmental parameters appearing in equation (5). In particular, it is possible to get a reliable value of *h* for the specific leaf geometry of the specimen. Newton's convection coefficient has a strong dependence on the geometry of the object and on its orientation to air flow and, more in general, in the case of leaves it is very difficult to obtain from a given theoretical formula.

Medical application

There are similarities in the application of thermographic analysis between plant and human studies since theskin is a conductive interface between an inner core and an outer, usually colder, environment. Thermal imaging can be used to show heat dissipation from the skin surface. From the physical point of view, skin can be represented as a thin monolayer of conductive material between the hot inner tissue where metabolic exoenergetic processes occor and the outside environment. Spectral emissivity has the same values, (>0,95) for animal and vegetable tissues and so the recording procedures are very similar because of the high water content (ε=0.98) of living tissues.

Good reviews of medical applications can be found in papers of Lahiri (2012) and Jones (1998). Sherman et al. used thermal imaging for evaluating skin temperature asymmetries between paired limbs of subjects (Sherman 1996). Thermography has been succesfully used in the diagnosis of diabetic neuropathy (Ring, 2010), vascular disorders (Bagavathappan et al., 2009), breast cancer detection (see below), thermoregulation studies (Ludwig et al., 2012, Formenti et al., 2013), dermatology, and the diagnosis of rheumatologic diseases and bowel ischemia (Brooks et al., 2000).

Historically temperature measurement has been used for clinical diagnosis because it has been proved to be a very good indicator of health (Tan et al., 2009). The human body is capable of maintaining a constant body temperature, despite being influenced by environmental heat transfer through convection, infrared radiation, transpiration and conduction. The core temperature has been preserved within a narrow range (Bouzida et al., 2009) and its regulation is essential for normal performance of the human body in healthy metabolism or agonistic exercise. Body temperature is closely controlled within precise limits. From a quantitative point of view the most important phenomenon employed in thermoregulation is evapotransipiration. One gram of water evaporated from skin surface can reduce more than 0.5kg of human tissue by 1°C.

Since the 1930's, the physiological role of infrared emission from human body has been well described and it was found that human skin can be considered as a blackbody radiator. With the advent of the new generations of solid state detectors, near and middle infrared spectral (NIR, MIR) regions are also used in medical applications. In the field of non destructive testing the application of IRT is mainly in surface and sub-surface defect detection and online monitoring of processes (Maldague, 2001). In these IRT applications, the surface thermal profile of tested objects show a typically gaussian distribution around a maximum value that can be referred to a hottest spot in the defect (see Figure 3).

Subsurface defects cause abnormal thermal conduction through the inner layers, which indicate the presence of those defects. In medical applications, by contrast, the clinical illness that manifests itself as abnormal thermal patterns on the skin surface can be monitored by taking into account the outer part of the object under examination. Biomedical applications of IRT are in general transmission inspection. Another aspect of medical image processing is asymmetry analysis, where temperature differences between the diseased and healthy body parts are analyzed. This technique is particularly useful in female breast cancer detection. In 1982, United States Food and Drug Administration approved IRT as an adjunctive tool for diagnosis of this kind of cancer. Kennedy et al (2009) presented a comparative study of IRT and other imaging techniques for breast screening and concluded that IRT provides additional functional information on the thermal and vascular condition of the tissues. Tumours generally have an increased blood supply and an increased metabolic rate which leads to localized high temperature spots over such areas, allowing them to be visualized by IRT. Apart from passive breast imaging, cold stimulation based imaging procedures are also used. Blood vessels, produced by cancerous tumours are simple endothelial tubes devoid of a muscular layer. Such blood vessels fail to constrict in response to sympathetic stimulus like a sudden cold stress and show a hyperthermic pattern due to vasodilatation. Gamagami (1986) has also shown that, hypervascularity and hyperthermia were visible in 86% cases of nonpalpable breast cancers.

In the diagnosis of diabetic neuropathy and vascular disorders, thermal imaging has proved to be a useful technique as both cause changes in skin surface temperature. Branemark et al. (1967) studied a number of diabetic subjects and found that all of them had abnormal temperature patterns in their feet and hands such as reduced temperature on the toes, metatarsal regions and fingers. Bagavathiappan et al. (2008) investigated diabetes mellitus subjects with vascular disorder and found that the temperature of the affected regions is higher than that of the unaffected regions. Cutaneous temperature discrimination could be also used for early diagnosis of diabetic subjects.

Skin diseases generally cause inflammation which in turn causes abnormal temperature pattern on the skin surface. IRT may thus be considered a suitable technique for studying skin diseases. IRT has been used for diagnosis of leprosy subjects and it has been reported that, cooler areas (like ear and nasal rim region) are heavily affected. Some authors (Flores – Sahagun, 2011) described a technique for indirect monitoring of skin blood perfusion, preoperative planning and post-operative monitoring and used IRT for diagnosis and analysis of basal cell carcinoma, which is the most common form of malignant skin cancer.

IRT can be also useful to identify and visualize possible asymmetries in the whole body, and to monitor the muscular development in order to correct posture. Figure 4 shows a typical thermal pattern of a human back, following long term exercise to correct the scoliosis.

Thermal radiation emitted by a surface is detected by an infrared camera and the intensity of the emitted radiation is converted to temperature. IRT can be static or dynamic. In the former case, instantaneous temperature distribution is monitored, whereas in the latter case, temporal variation of temperature distribution is monitored. A series of thermal images are acquired, which constitute a time series in temperature. In a review article Jones and Plassmann (2002), discussed the importance of image processing techniques in IRT and the need to form a common database of normal thermal images of all possible parts of a human body has been identified. Ring (2010) have discussed the technical challenges for constructing a digital medical IR image database and they have developed an Anglo-Polish reference database of medical thermal images. For comparison of multiple thermal images, several groups have used a method based on the comparison of various regions of interest (ROI) in multiple thermal images where the ROIs are semi automatically aligned (Merla, 2010).

Image acquisition may be standardized with the use of software masks (automated or manual), which provide an outline of the regions of interest. A series of such automated masks have been developed and in particular four operations are of interest: (i) subtraction of the background in each frame of interest, (ii) construction of a temperature time profile at a chosen point or regions, (iii) spatio-temporal projections of image sequences and (iv) calculation of the time derivative of temperature data. Fourier analysis is another useful image processing technique, which enables isolating signals of a particular frequency. Another aspect of medical image processing is asymmetry analysis, where temperature differences between the injuried (Lahiri, 2012) and contralateral healthy body parts are analyzed (Ferreira et. al, 2008).

In this field IRT can be used to monitor the post-trauma evolution. The anamolous thermal pattern of the right knee of a volleyball player after a injury on a *Ligamenta cruciata genus (LCA)* can be seen in Figure 5, comparing right and left knees.

Thermal imaging is particularly useful in female breast imaging. The latest development in thermal image processing applied to a moving subject, as in medical applications, is the introduction of hitherto military oriented automatic target recognition algorithms (Ludwig et al., 2007). The introduction of this highly sophisticated and accurate technique is expected to result in a paradigm shift in infrared image based computer aided diagnosis. Artificial neural networks and fuzzy logic will play an important role in the development of this technology. Brioschi et al. developed a method for fusion of 2 dimensional infrared images, which lack information about local anatomy, with 3 dimensional magnetic resonance imaging for multi-level diagnosis (Brioschi et al., 2002).

Thermoregulation

Blood circulation is the principle fast mechanism of heat transfer inside human body. A relationship between heat radiating from the skin surface and the surrounding blood flow, has been described by the following equation, which is known as Pennes bio-heat equation.

$$Q_m = C_b W_b (T_a - T) - \nabla^2 T \qquad (6)$$

Where Q_m is the volumetric metabolic rate of the tissue, k is the thermal conductivity of tissue material, C_b is the specific heat capacity and W_b is the mass flow rate of blood per unit volume of tissue, T is the unknown tissue temperature and T_a is the arterial temperature. Based on this bio-heat equation, various numerical models have been developed. Apart from a basic understanding of the problem, in principle, such numerical models serve both to predict skin temperature on the basis of internal heat generation and solving the inverse bio-heat equation to estimate the temperature of internal organs by measuring skin temperature. Numerical modelling also helps in optimization of the experimental parameters. Cooling processes are effective methods to improve the sensitivity of thermal images. Figure 6, shows typical thermal images of the left hand of a normal subject with a finger tendon injury, after 10 seconds and 1 minute of water cold immersion. The injured finger can be clearly seen because the cold stimulation increases the sensitivity of thermal images and renders subtle thermal signatures to be clearly discernible.

During exercise, metabolism and flexing of muscles are the main sources of heat in body core (Jones, 1998). Thereafter, the heat is transferred from the core towards the outer parts of the body by blood flow through blood vessels. The blood gains heat from the core of the body and loses heat at the peripheral parts, especially the limbs and head, where the ratio of surface area to volume is the most favourable for heat dissipation. Monitoring this process can give useful knowledge about the physiology of thermoregulation. IRT can be useful to recognise the pattern of the superficial vasocirculation. Venous blood even if colder than arterial blood can be detected where the vessels are not hidden by fat. This can be seen both in normal condition (Figure 7).or after heavy exercise involving the femoral quadriceps (squat) as in Figure 8.

The dynamics of surface temperature distribution is governed by two main factors, convection by blood flow in the surface layer and from deeper blood vessels and sweat evaporation from the cutaneous layer. Thermography is an effective tool for monitoring thermoregulation processes. In the majority of healthy subjects, the temperature of the hands, feet, neck and facial regions increases after immobilization. This is because immobilization is generally followed by relaxation and blood redistribution, which causes an increase of skin temperature. Spontaneous periodic variations, due to changes in the sympathetic nervous system and blood flow, are also observed through temperature profiles of hands and feet (Zontak et al., 1998). Oscillations having a period of less than 4 minutes are related to capillaries whereas those with higher periods are due to arterio-venous anastomoses. Vainer (2005) in his monograph on cutaneous perspiration, used MIR to study the sweating procedure. Some authors found that the low temperature region surrounding the secretory duct of each individual sweat gland is axissymmetric.

An insightful discussion correlating sweating and thermoregulatory mechanism is also presented. Bouzida et al. (2009) studied thermoregulatory mechanisms by two approaches: blood flow modulation and cold stress. In the first approach, blood pressure is modulated, within the systolic and diastolic values, by using a suitable mechanical arrangement. In the second approach, a cold stress was applied by placing the left hand on a cold metal surface and the temperature dynamics of both hands were observed using a thermal camera. They observed that, after a long steady state period of 85 seconds the temperature on the stimulated hand decreased while the temperature on the other hand increased. This phenomenon can be attributed to the fact that thermoregulatory mechanism responds in such a way that the core temperature remains undisturbed.

Emissivity

Emissivity of human skin is reported to be almost constant and its value is 0.98 ± 0.01 for wavelength range of 2–14 μm (Jones et Plassmann, 2002). Steketee (1973) reported that the emissivity of black, white or burnt skin is the same and it is independent of the nature of the experiments, i.e. in vivo or in vitro. The errors in surface temperature measurement are small for viewing angles up to 45°. The emissivity value of a surface changes not only with respect to wavelength λ but also from the viewing angle Φ. A useful relationship is given by the following equation:

$$\varepsilon_{\phi\perp} = 1 - \left[\frac{1}{2}\left(\frac{\beta - \cos\phi}{\beta + \cos\phi}\right)^2\right]\left[1 + \left(\frac{\beta\cos\phi - \sin^2\phi}{\beta\cos\phi + \sin^2\phi}\right)^2\right]$$

where $\beta = \sqrt{n^2\ sin^2\ \Phi}$ and n is the refractive index of the material. For medical applications, surface curvature is not a problem.

Conclusions

As abnormal thermal patterns are easily detectable by IRT, an early diagnosis is possible from these thermal images. Thermographic findings are usually compared with other clinical findings to assess for possible correlations. Even if thermographic methods are nonspecific and sometimes dependent heavily on background and surrounding environment, there are a number of reasons for which IRT has found wide acceptance among the medical community. This is primarily because IRT is a remote, non-contact and non-invasive technique.

REFERENCES

1) Bagavathiappan S., Saravanan T., Philip J., Jayakumar T., Raj B., Karunanithi R., Panicker T.M., Korath P. and Jagadeesan K. (2008) Investigation of peripheral vascular disorders using thermal imaging. The British Journal of Diabetes and Vascular Disease, **8,** 102–104.
2) Bagavathiappan S., Saravanan T., Philip J., Jayakumar T., Raj B., Karunanithi R., Panicker T., Korath M.P. and Jagadeesan K. (2009) Infrared thermal imaging for detection of peripheral vascular disorders. Journal of Medical Physics **34,** 43–47.
3) Bajons P, Klinger G, and Schlosser, V. (2005) Determination of stomatal conductance by means of infrared thermography. Infrared Physics & Technology, **46,** 429-439.
4) Bouzida, A., Bendada, X.P. and Maldague, L.J. (2009) Visualization of body thermoregulation by infrared imaging. Journal of Thermal Biology, **34**, 120–126.
5) Boccara, M., Boue, C., Garmier, M., De Paepe, R. and Boccara, A.C. (2001) Infrared thermography revealed a role for mitochondria in pre-symptomatic cooling during harpin-induced hypersensitive response. Plant J. **28,** 663–670.
6) Branemark, P., Fagerberg, S., Langer, L. and Soderbergh, J.S. (1967) Infrared thermography in diabetes mellitus. Diabetologia, **3** 529–532.
7) Brioschi, M.L., Colman, D., and Neto H.M. (2002) Fusing IR and magnetic resonance (MR) image. Journal of Korean Medical Thermology, **2**, 57–58.
8) Brooks, J.P., Perry, W.B., Putnam, A.T. and Karulf, R.E. (2000) Thermal imaging in the detection of bowel ischemia. Diseases of the Colon and Rectum **43** 1319–1321.
9) Chaerle, L., De Boever, F. and Van Der Straeten, D. (2002) Infrared detection of early biotic and wound stress in plants. Thermology Int., **12**, 100–106.
10) Chaerle, L., Hagenbeek, D., De Bruyne, E., Valcke, R. and Van Der Straeten, D. (2004) Thermal and Chlorophyll-Fluorescence Imaging Distinguish Plant-Pathogen, Interactions at an Early Stage. Plant Cell Physiol., **45**, 887–896.
11) Ferreira, J.J.A., Mendonça, L.C.S., Nunes, L.A.O., Andrade Filho, A.C.C., Rebelatto, J.R. and Salvini, T.F. (2008). Exercise associated thermographic changes in young and elderly subjects. Ann. Biomed. Eng. **36**, 1420–1427.
12) Flores-Sahagun, J.H., Vargas, J.V.C. and Mulinari-Brenner, F.A. (2011) Analysis and diagnosis of basal cell carcinoma via infrared imaging. Infrared Physics & Technology, **54**, 367–378.

13) Formenti, D., Ludwig, N., Gargano, M.; Gondola, M.; Dellerma, N., Caumo, A. and Alberti, G. (2013) Thermal Imaging of Exercise-Associated Skin Temperature Changes in Trained and Untrained Female Subjects. Annals of Biomedical Engineering , DOI: 10.1007/s10439-012-0718-x.
14) Gamagami, P. (1986) Atlas of Mammography: New Early Signs in Breast Cancer, Blackwell Science, United Kingdom.
15) Jones, H.G. (1999) Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant, Cell and Environment, **22**, 1043-1055.
16) Jones, H.G., Stoll, M., Santos, T., de Sousa, C., Chaves, M.M. and Grant, O.M. (2002) Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. Journal of experimental botany, **53**, 2249-2260.
17) Jones, B.F. (1998) A reappraisal of the use of infrared thermal image analysis in medicine. IEEE Transactions on Medical Imaging, **17**, 1019–1027.
18) Jones, B.F., and Plassmann, P. (2002) Digital infrared thermal imaging of human skin. IEEE Eng. Med. Biol. Mag., 41–48.
19) Hellebrand, H.J., Beuche, H. and Linke, M. (2002) Thermal imaging. A promising high-tec method in agriculture and horticulture. In: J. Blahovec and M. Kutílek, Editors, Physical Methods in Agriculture — Approach to Precision and Quality, Kluwer Academic/Plenum Publishers, New York , pp. 411–427.
20) Kennedy, D., Lee, T. and Seely, D. (2009) A comparative review of thermography as a breast screening technique. Integrative Cancer Therapies, **8**, 9–16.
21) Lahiri, B.B., Bagavathiappan, S., Jayakumar, T. and Philip J. (2012) Medical applications of infrared thermography: A review. Infrared Physics & Technology, **55**, 221–235.
22) Leinonen, I. and Jones H.G. (2004) Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. J. Exp. Bot., **55**, 1423-1431.
23) Ludwig, N., Cabrini, R., Faoro, F., Gargano, M., Gomarasca, S., Iriti, M., Picchi, V. and Soave, C. (2010) Reduction of evaporative fluxe in bean leaves due to chitosan treatment assessed by infrared thermography. Infrared Physics & Technology, **53** 65-70.
24) Ludwig, N., Gargano, M., Luzi, F. and Verga M. (2008) Applicability of infrared thermography to stress state detection in study on rabbit welfare. In: Procedings of Advanced Infrared Technology and Applications, 425-430
25) Ludwig, N.G., Gargano, M., Formenti, D., Bruno, D., Ongaro, L. and Alberti G. (2012) Breathing training characterization by thermal imaging: A case study. Acta of Bioengineering and Biomechanics, 41-47.
26) Maldague, X. (2001) Theory and Practice of Infrared Technology for Nondestructive Testing. First edition. John Wiley and Sons, New York.
27) Merla, A., Mattei, P.A., Di Donato, L. and Romani G. L. (2010). Thermal imaging of cutaneous temperature modifications in runners during graded exercise. Ann. Biomed. Eng., 158– 163.
28) Milazzo, M., Ludwig, N., Villani, S., Soave, C. and Cicardi, C. (1994) Applicability of IR thermography to measurement of leaf transpiration. Atti Fondazione G.Ronchi IL-1 Firenze, 171-180.
29) Morandini, P., Murgia, I., Campi, L., Gomarasca, S., Villani, S., Ludwig, N. and Soave C. (1996) Resistance to phytotoxins: developing selection methods based on the physiological action of the toxin. PETRIA, giornale di patologia delle piante, 133-142.
30) Mustilli, A.C., Merlot, S., Vavasseur, A., Fenzi, F. and Giraudat, J. (2002) Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell **14**: 3089–3099.
31) Nobel, P.S. (1983) Biophysical plant physiology and ecology. WH Freeman and Company, San Francisco, 608-612

32) Pereira, L. S., Oweis, T. and Zairi, A. (2002) Irrigation management under water scarcity. Agric. Water Manag. **57**, 175-206.
33) Ring, R. (2010) Thermal imaging today and its relevance to diabetes. Journal of Diabetes Science and Technology. **4**, 857–862.
34) Sherman, R.A., Wideman, A.L. and Karstetter K.W. (1996) Comparative effectiveness of videothermography, contact thermography, and infrared beam thermography for scanning relative skin temperature. Journal of Rehabilitation Research and Development., 377–386.
35) Skaracis, G.N. and Biancardi, E. Breeding for Cercospora resistance in sugar beet. In Cercospora beticola Sacc. Biology, Agronomic Influence and Control Measures in Sugar Beet. Edited by Asher, M.J.C., Holtschulte, B., Molard, M.R., Rosso, F., Steinrücken, G. and Beckers, R. (2000) Advances in Sugar Beet Research Series, 177–195.
36) Steketee J., Spectral emissivity of the skin and pericardium, Physics in Medicine & Biology 18 (1973) 686–694.
37) Tan, J.H., Ng, E.Y.K., Acharya, U.R. and Chee C. (2009) Infrared thermography on ocular surface temperature: a review. Infrared Physics & Technology., 97–108.
38) Vainer B.G., FPA-based infrared thermography as applied to the study of cutaneous perspiration and stimulated vascular response in humans, Physics in Medicine & Biology 50 (2005) R63–R94.
39) Zontak, A., Sideman, S., Verbitsky, O. and Beyar, R. (1998) Dynamic thermography: analysis of hand temperature during exercise. Ann. Biomed. Eng., 988–993.

Figure 1. Cucumber leaf, green-blue spots are due to a local treatment with paralizing abscisic acid stomata in open position during a water stress condition.

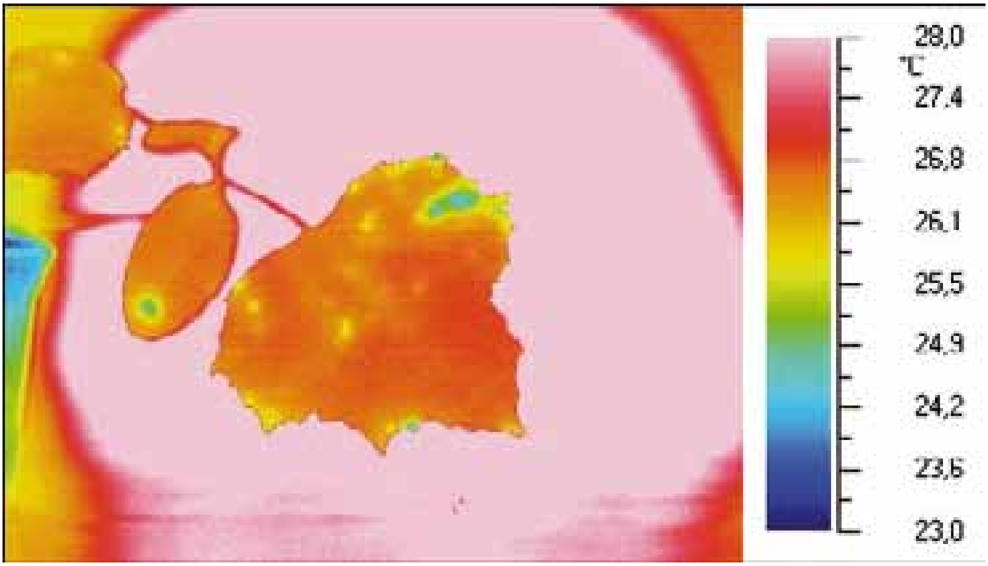


Figure 2. Correlation evaporation flux from leaf surface vs. cooling.

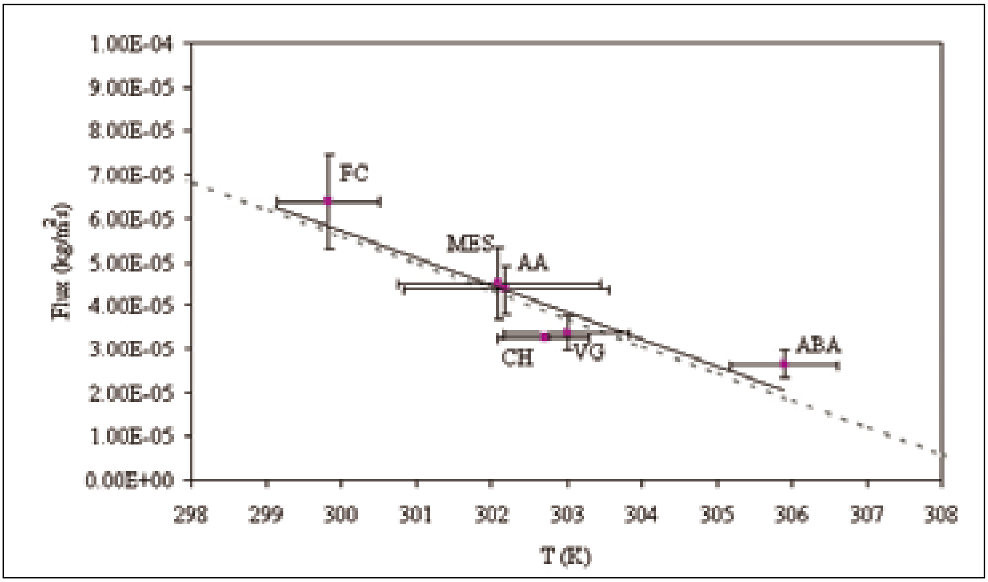


Figure 3. Typical Gaussian pattern of temperature distribution in a visualisation of defects on metallic surface by active thermography test.

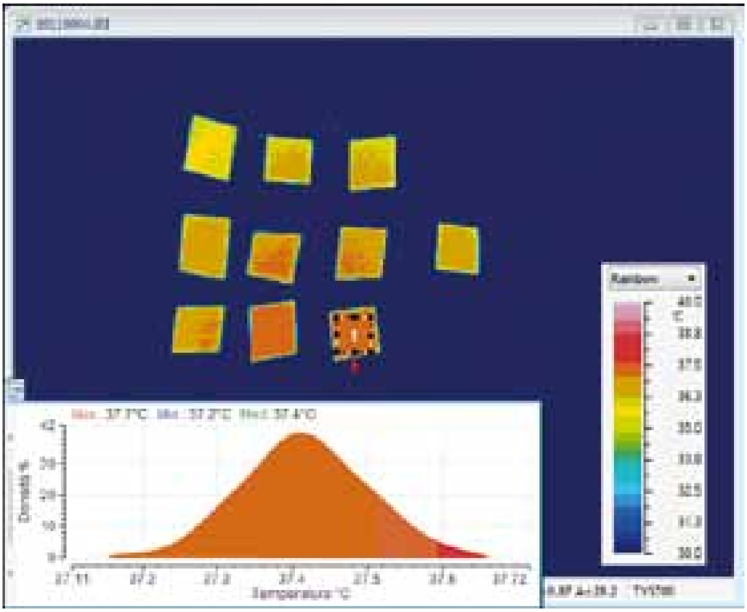


Figure 4. Tthermographic evidence of controlateral thermal anomalies in a trained male subject (34 y.o.) with scoliosis, left part of the mid-back show a wide colder area.

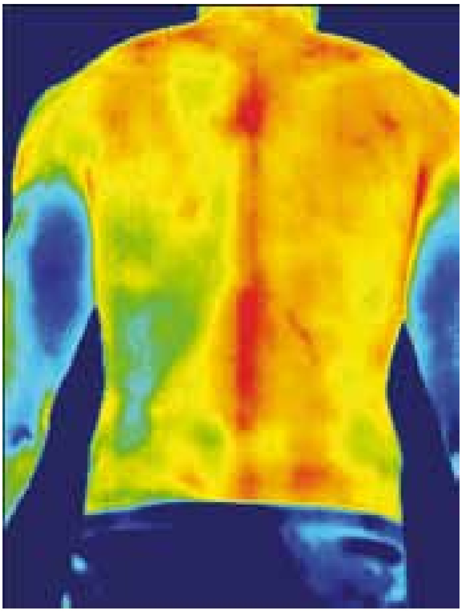


Figure 5. Thermographic evidence of trauma in right knee (on left) on a volley player male subject (23 y.o.) after one month from LCA fracture.

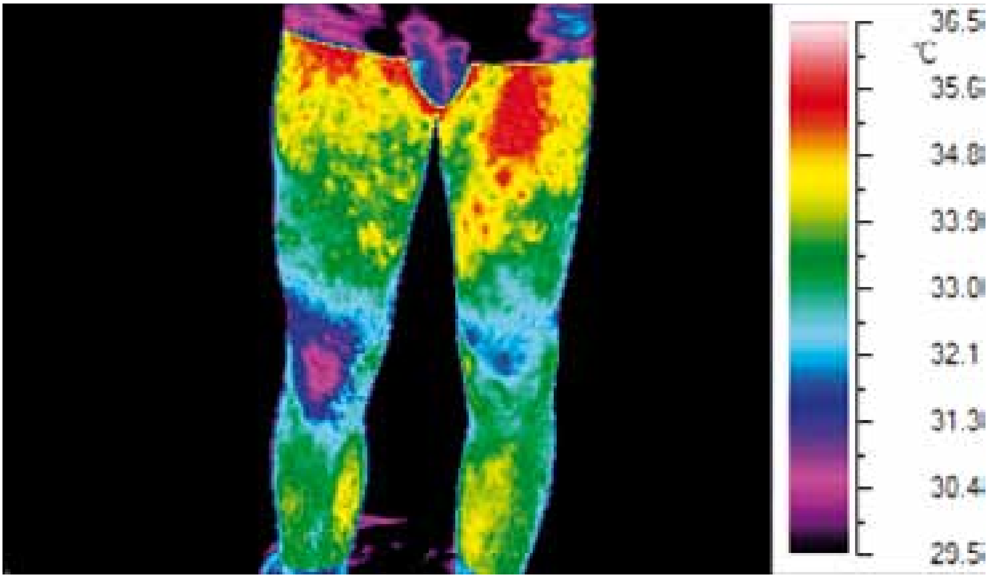


Figure 6. Typical IR images of the left hand of a normal subject with finger tendon injury (trained male, 19 y.o.), after immersion in cold water. Left after 10 seconds right after 1 minute. The injured finger can be clearly visualized when circulation recover the original temperature on the healthy fingers.

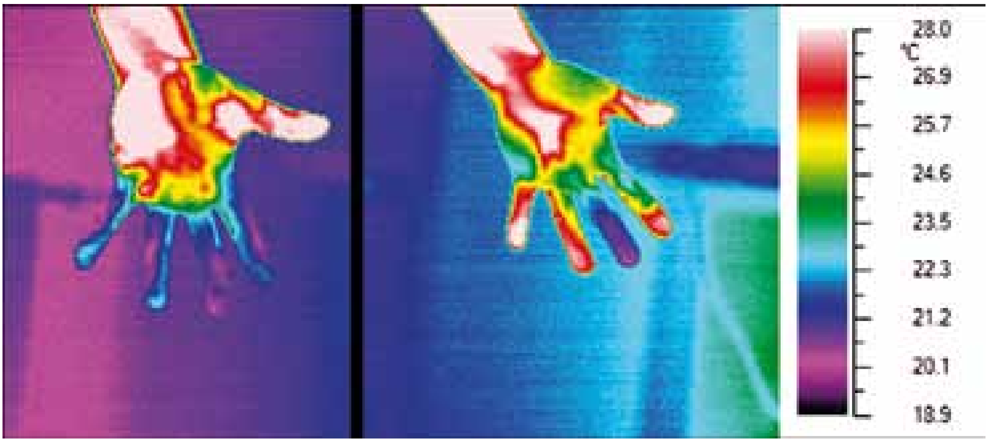


Figure 7. Thermographic evidence of subcoutaneous vaso circulation in a male healty subject (27 y.o.).

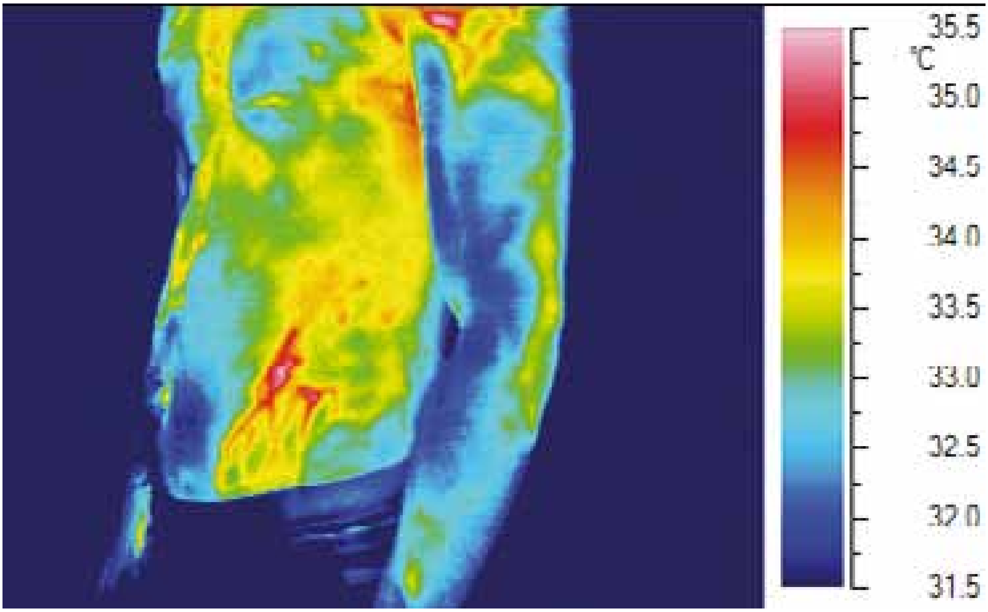
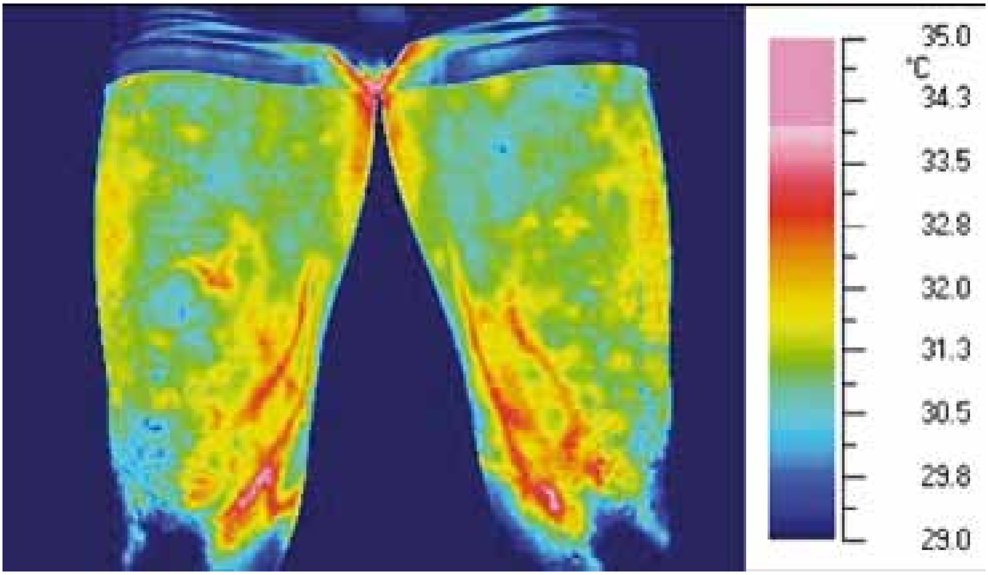


Figure 8. Thermographic evidence of subcoutaneous vasal circulation in a male well trained subject (23 y.o.) during squat exercice.



THERMAL IMAGING THEORY

VERONICA REDAELLI[1], SIMONE CAGLIO[2]

[1] University of Milan, VESPA Department, Italy
[2] Deralab S.r.l.– Seregno, Italy

ABSTRACT

Infrared thermography is a technique that allows the remote measurement of the surface temperature of an object. A thermal imaging camera provides colors images, where each color corresponds to a specified temperature. Modern cameras provide real-time thermal images accurate to hundredths of a degree which allow the smallest details to be seen. The difference in behavior of a real body with respect to the black body is indicated by the parameter spectral emissivity. It is important to note that in animal tissues the large concentration of water in general makes the emissivity high (more than 0.80). Some parts of the body not covered by hair are the only areas on which it appears possible to read the correct surface temperature of the animal. It is not possible, in principle, to ascribe a specific pathology to a specific color and it is only in the context of a more general history of the disease or of the state of the animal that the presence of a "thermal anomaly" can be connected to the correct physiological phenomenon. It is important to note that in thermographic analysis each object should be studied in relation to its characteristics and with the surrounding environment and each species has some unique technical problems which may affect the success of the measurements.

Key words

Thermography, infrared radiation, black body, emissivity, false color image.

Infrared thermography is a technique that allows the remote measurement of the surface temperature of an object. A thermal imaging camera provides colors images, where each color corresponds to a specified temperature (Figure 1).

This is possible because thermography detects the infrared (IR) radiation spontaneously emitted from all bodies with a temperature more than -273°C and which has a wavelength between 0.75 μm and 1000 μm (Sparrow et al., 1978).

In physics, a black body is an ideal object that absorbs all incident electromagnetic radiation without reflecting or transmitting any energy. As the body is absorbing all of the incident energy and not dispersing in another way, because of the energy conservation principle, the black body will have to re-radiate exactly the same amount of energy absorbed.

The intensity and frequency of the emitted radiation are closely related to the temperature of the source through well known laws of physics. In the late nineteenth century, physicists Stefan and Boltzmann established first experimentally, and then theoretically, the relationship existing between the quantity of energy emitted by a black body and its temperature. The energy (E) is proportional to the fourth power of the absolute temperature (T) and this is called the law of black body emission (1):

$$E = \sigma T^4 \quad (1)$$

where $\sigma = 5{,}67\ 10^{-8}$ W/m^2 K^4 is the Boltzmann constant.

Furthermore, Wien's law describes the relationship between the peak wavelength of emission of a black body and its temperature T(2):

$$\lambda_{max}T = 2{,}9\ 10\ \text{m K} \qquad (2)$$

The emission peak moves towards shorter wavelengths as the temperature increases, so a material superheated to about 3000 K has a maximum emission in the area of near infrared (1 μm), while objects at room temperature (300 Kelvin, 27 °C) have the corresponding emission peak at 10 μm (Figure 2).

Tungsten lamps (approximately 3000 K), produce radiation both in the visible and in the near infrared spectrum. The sun is similar to a large sphere of hot gas whose outer surface has a temperature of about 6000 Kelvin and emits at a wavelength which corresponds to the daytime lighting on Earth. The coldest bodies at room temperature, about 300 Kelvin, emit just infrared radiation (Figure 2).

Using these laws, modern thermal imaging cameras provide real-time thermal images accurate to hundredths of a degree which allow the smallest details to be seen. Modern detectors are of extremely high sensitivity and coupled to the excellent quality of the optical systems employed ensure the sensitivity of current thermal imaging cameras is capable of producing high resolution images. The sensitivity ad resolution of any particular camera depend upon the size and specification of the detector.

The thermal imaging camera is very similar to an optical camera but has optics for infrared instead of the optical elements commonly used for general photography. Optical glass is not suitable for the purpose since it has a high absorbance in the wavelengths typical of the infrared. Germanium is the commonly used material because it is transparent to infrared radiation. By contrast, the atmospheric transparency to infrared radiation is one of the primary factors that make the thermographic technique possible. Figure 3 shows the Earth's atmospheric transparency to electromagnetic radiation. Transparent areas (in white) are called atmospheric windows and dark areas are portions of the spectrum where the atmosphere is partially or totally opaque to the passage of radiation, due to molecular interference (e.g. water molecules) selectively absorbing those wavelengths (Peixoto et al., 1992).

Three "windows" can be seen in Figure 3: the first one is in the visible/near IR spectrum, the second one in the middle IR (3-5 μm) and the third one in the far IR (8-14 μm). The position of these atmospheric transparency bands has determined the direction of technological improvements allowing us to detect infrared radiation.

The detector of the camera, on which the IR radiation is focused, transforms the incident energy into an electrical signal to be supplied to the amplification circuit. The detector output signal is amplified and converted from analog to digital and sent to a computerized system that displays images and processes the data that has been collected. The main distinction between IR devices is the spectral range in which they work (in relation to the 3 atmospheric windows). As regards the thermal infrared (second and third window) we can distinguish two types of instrument, one working in the short wave range (SW) and the other in the long wave range (LW). SW instruments generally comprise solid state detectors systems that operate in the spectral range 3-5 μm. This kind of device is extremely sensitive but the amount of radiation emitted by a body at room temperature is minimal in this spectral band (Figure 2). Instead, microbolometric detectors are employed in LW devices (LW, 8-14 μm), where the detectors lower sensitivity is compensated by the greater amount of energy available and by the almost total absence of solar radiation disturbance (Maldague, 2001).

The use of solid state detectors requires a cooling system to maintain a low background noise and improve performance. Such cooling systems lead to an increase in the overall dimensions of te device and a decrease of apparatus robustness. The uncooled microbolometric detectors are therefore the most widely used for measurements on site, as for animals.

In addition to the type of detector, another important characteristic is the ability to distinguish between two neighbouring points, called spatial resolution, which is usually expressed in mrad. An infrared thermography (IRT) IRT system with a spatial resolution of 1.4 mrad is able to distinguish the temperature of two points 1.4 mm apart at a distance of 1 m. A modern thermal imaging camera with a 320x240 pixel microbolometric detector and 35 mm standard optical lens, allows a spatial resolution of a few centimetres at 10 m distance. At that distance the smallest object that can correctly have its temperature measured must be 14 mm wide (1.4x10). At a distance of 20 m the object size must be 28 mm wide. Additional lenses, such as wide-angle lenses, telephoto lenses, macro lenses are available to increase this feature.

Another critical factor in achieving correct temperature measurements of a surface is the emissivity. Only the so-called *black body* behaves exactly according to the laws (1) and (2) above (Incropera et al., 2006). Real objects, in addition to absorbing the incident radiation, will reflect and transmit a part of that radiation and consequently the intensity of energy emitted will be lower, than that of a black body (Figure 4).

The difference in behavior of a real body with respect to the black body is indicated by the parameter *spectral emissivity,* ε_λ , defined as the ratio between the energy (W) emitted by a body at a temperature (T) and the energy that an ideal black body emits ($W_{blackbody}$) at the same temperature:

$$\varepsilon_\lambda = \frac{W(T)}{W_{blackbody}(T)} \quad (3)$$

It follows that the maximum emissivity value is 1 (black body), while the minimum is 0 (a totally reflecting body). A surface characterized by a high emissivity value (close to 1) has a low capacity to reflect and vice versa; most of the organic and amorphous materials belong to the first category, while metals in particular belong at the second one.

It is important to note that in animal tissues the large concentration of water in general makes the emissivity high (more than 0.80), so the presence of errors due to reflected radiation is minimal. Other metal objects or special keratinic materials (scales, bones, nails), characterized by low emissivity, may reflect the energy present in the environment, providing both erroneous measures of temperature and thermal anomalies (Figure 5).

Emissivity values for most materials are available in reference sources and a modern thermal imaging camera already provides the correct temperature of the analyzed surface by setting the correct emissivity value, thus taking advantage of equation (4):

$$\sigma T_{tvs}^4 = \varepsilon\sigma T^4 + (1-\varepsilon)\varepsilon^{env}\sigma T_{env} \quad (4)$$

where:

- T_{tvs} is surface temperature
- ε is surface emissivity
- $\varepsilon_{env}\,\sigma\,T^4_{env}$ is the energy radiated from the environment (where environmental emissivity = 1) relative to the temperature at which the measurement is carried out, which is generally measured by a thermocouple integrated to the thermal imaging camera.

The advantage of the thermographic technique is the representation of the results in the form of digital images which can subsequently be appropriately edited to meet the specific requirements using image processing software. Thermal images are initially represented in grayscale, each level corresponding to the different intensity of radiation detected (Pajani, 1989). The *false color* images are obtained by ascribing, to each gray level, a color specifically chosen to better highlight thermal information.

By example, with reference to non invasive tests on animals, Figure 1 shows the thermography of a rabbit. On the right of the image a scale shows the different colors in relation to the respective temperatures. The bottom is purple because it is characterized by a temperature close to 26 ° C, as shown in the scale. Rabbit fur appears with a temperature slightly higher than that of the surrounding environment, showing its thermal insulation, which limits heat loss from the body. Some parts of the body not covered by hair, such as nose, ears and eyes, are the only areas on which it appears possible to read the correct surface temperature of the animal; In some specific locations, for example the ear and eye, some anatomical details are identifiable such as differences in blood flow. It is not possible, in principle, to ascribe a a specific pathology to a specific color and it is only in the context of a more general history of the disease or of the state of the animal that the presence of a "thermal anomaly" can be connected to the correct physiological phenomenon.

Considering the practical application of the IRT techniques, they can be divided first of all into passive and active. Passive measurements are taken on objects found in the environment. Active thermography submits bodies to a heating or cooling process and investigate the process of returning to normal thermal conditions.

To increase the information obtainable that a single thermal image could not highlight, it can be useful to develop dynamic sequences which are then processed with special algorithms studying the temporal evolution of the images.

It is important to note that in thermographic analysis each object should be studied in relation to its characteristics and with the surrounding environment. For animals, each species has some unique technical problems due to the type of subject (size, fur, behavioral characteristics, type of housing, etc.), which may affect the success of the measurements. This fact does not allow the definition of standard operating techniques that might be usable in all situations. Often, only the experience and knowledge of the species being studied allows the identification of the best solutions by adapting the methods to the specific needs.

REFERENCES

1) H.S.Carslaw, J.C.Jaeger, Conduction of heat in solids , Oxford U.Press, 1959.
2) G.Gaussorgues, Infrared Thermography , Chapman & Hall, 1994.
3) Frank P. Incropera, David P. Dewitt, Theordore L. Bergman, Adrienne S. Lavine, Fundamentals of Heat and Mass Transfer , John Wiley & Sons Inc, New York (2006).
4) X. Maldague, Theory and Practice of Infrared Technology for Nondestructive Testing, first ed., John Wiley and Sons, New York, 2001.
5) D.Pajani, Mesure par Termographie Infrarouge , ADD Editeur, Chatenay Malabry,1989.
6) J.P. Peixoto, A.H. Oort, Physics of Climate. American Institute of Physics, 1992.
7) E.M. Sparrow, R.D. Cess, Radiation heat transfer. Hemisphere Pub. Corp., 1978.

Figure 1: Infrared image of a rabbit.

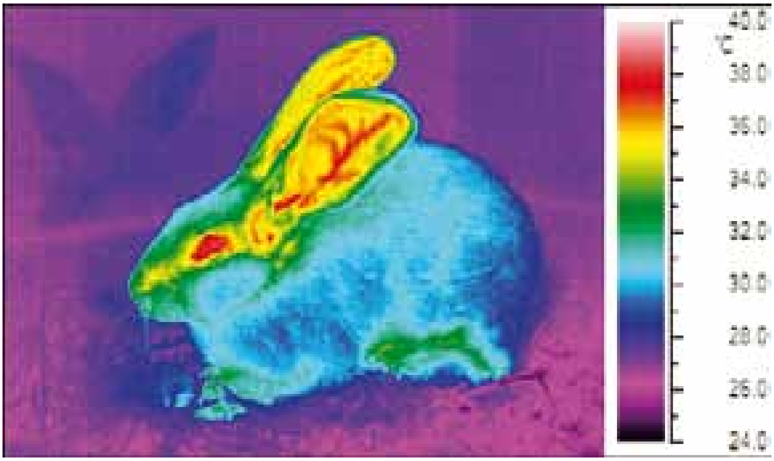


Figure 2: The peak of emission moves towards shorter wavelengths as the temperature increases. Wavelength on X axis, radiance on Y axis.

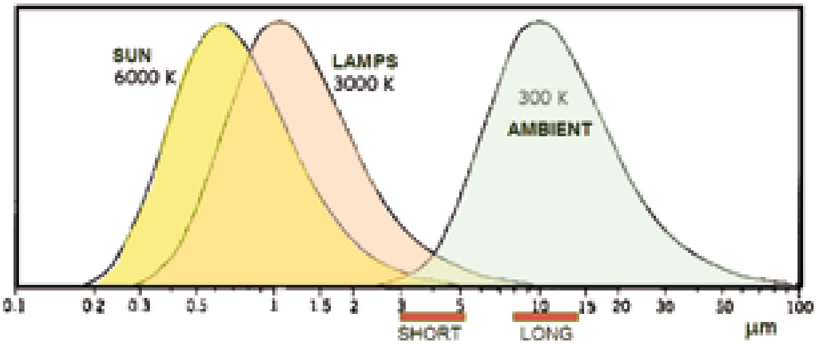


Figure 3: Atmospheric windows; wavelength (mm) on X axis, absorbtion (%) on Y axis (J.P. Peixoto, A.H. Oort, 1992).

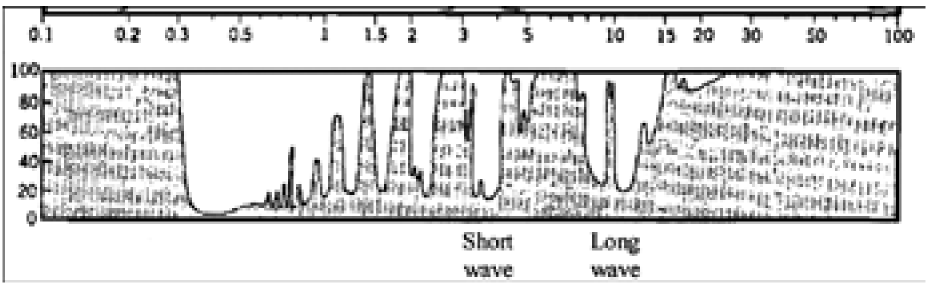


Figure 4: Difference in behavior of a real body (orange) with respect to the black body (yellow).

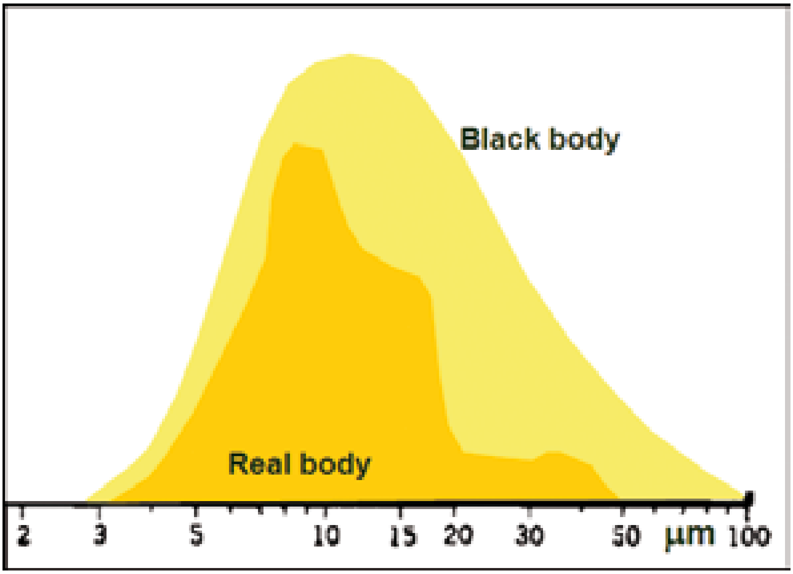
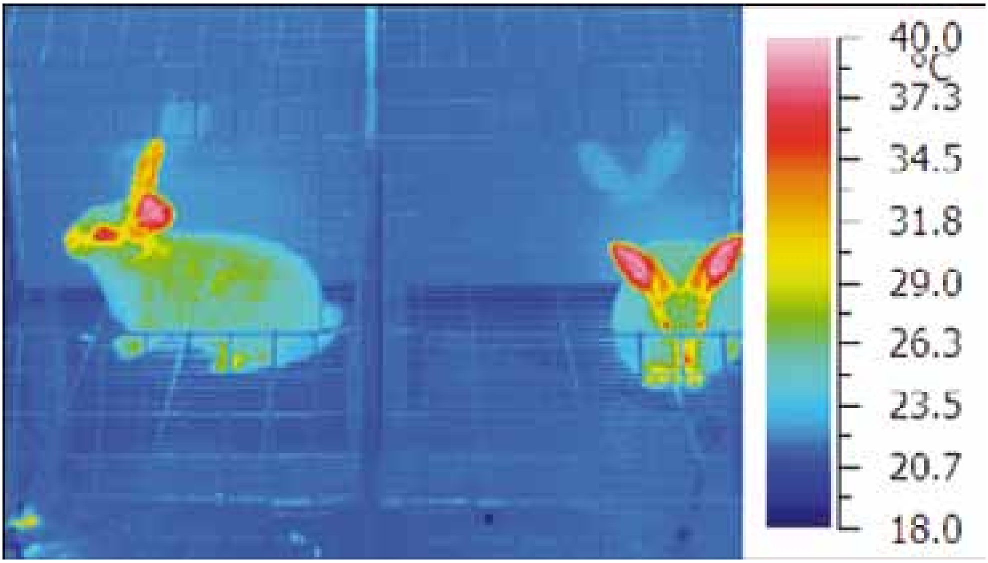


Figure 5: The two rabbits partially visible on the background (light blue) are artefacts due to the low emissivity and therefore the high reflectivity of the metal cage.



THERMAL IMAGING IN PHYSIOLOGY: THEORETICAL AND PRACTICAL CONSIDERATIONS

MALCOLM A. MITCHELL

SRUC, the Roslin Institute Building, Easter Bush, Midlothian, EH25 9RG, UK.

ABSTRACT

There are a large number of possible applications of thermography or thermal imaging to the study of human and animal physiology. The examples provided in this chapter constitute only a small proportion of the potential catalogue of physiological systems and responses that might be examined by thermography. The most obvious areas of application are determination of body temperature, regional temperature and heat exchange, characterisation of physiological status and stress, identification of altered metabolic state and muscle activity and changes in vasomotor tone and microcirculation in the skin. Any physiological or metabolic system or response that will influence heat production, transfer and loss may cause changes in delivery of heat to the animal surface. Such changes can be detected by thermal imaging and quantified and these measures related to the underlying mechanisms and processes. The practical and theoretical considerations, constraints and limitations described in the current chapter and elsewhere in this publication must inform those intending to employ successfully the methodology in physiological studies.

Key words

Infrared thermography, physiology, body temperature, heat exchange, thermoregulation.

Temperature is the most frequently measured physical quantity, second only to time and infra-red technology has been successfully employed to measure temperature in many scientific disciplines (Kastberegr and Stachl 2003). Thermal imaging, infrared thermography or thermal video are, by definition, methodologies for the measurement of temperature by detection of radiation in the infra-red component of the electromagnetic spectrum. The fundamental physical principles underlying the emission, absorption, reflection, transmission and detection of this radiation and the conversion of detected incident radiation into temperature measurements are described in detail elsewhere in this publication. The importance of understanding the relevance of emissivity of target biological surfaces is emphasised along with assurance that all physiological thermal imaging is undertaken with full cognisance of the physical limitations and capabilities of the technology. The thermal images or thermograms generated by thermal cameras (imaging devices) are effectively thermal maps of the surface of the target object displayed and stored with the addition of "false colour" to identify areas of different temperatures. The true temperatures and their distributions are contained within the digital images as absolute values in pixels and can be extracted for subsequent detailed analysis. In experimental human and animal physiology thermal imaging will allow characterisation of the temperature of the surfaces of live subjects and the absolute values of these temperatures and their distributions may be related to underlying physiological, metabolic and behavioural processes and mechanisms. In order to interpret thermograms of human and animal subjects, either for whole organisms or for specific surface regions, it is essential to understand the theoretical principles that govern the temperature distributions and heat exchange at the surface, skin or coats of the subjects. It is also necessary to understand how these thermal distributions might be influenced by physiological and metabolic processes elsewhere in the animal as well what effects responses and adaptations to various challenges and pathologies may have upon peripheral and surface thermal profiles.

What determines the surface temperature of the subject?

The basic principles of thermal exchange governing heat gain and loss by animals and man have been reviewed extensively (e.g. Monteith 1973; Monteith and Mount 1974). An understanding of these physical principles has allowed the development and application of sophisticated models describing and quantifying thermal exchange between animals and their environments through conduction, convection, radiation and evaporation (Gebremedhin 1985; McArthur 1987, 1990; Turnpenny et al 2000 a and b). Obviously heat exchange will take place at the surface of the animal i.e. at the skin or coat or within the respiratory tract and it is the temperature of these surfaces that may be visualised by thermal imaging. It is therefore pertinent to consider what factors determine the surface temperatures of the subjects. An initial consideration is the difference in the nature of the thermal exchange surfaces of different subjects. Clearly, whilst the skin represents a boundary for thermal exchange the nature of the skin e.g. its thickness, structure (e.g. the presence of subcutaneous fat, a cuticle, scales, corrugations) and/or the addition of pelage or plumage (hair, coat, wool, fur or feathers) will profoundly affect heat exchange between an animal an its surroundings and therefore, skin temperature.

Thermography may be applied to all classes and species of animals. Thus, it is possible to produce and analyse thermograms for insects, amphibia, reptiles, birds and mammals taking account of the differences in anatomy, morphology, metabolism and physiology exhibited by the various classes. For the purposes of the current short review the focus will be upon mammalian and avian species but many of the principles outlined may be usefully applied to other classes of animal. In mammals and birds the regional distributions of insulating layers or cover (e.g. pelage or plumage) will give rise to differential distributions of surface temperatures in these subjects. Where hair, coat or feather cover is most dense these heavily covered areas will appear in thermograms as the temperature of the outer layers or surface of the pelage or plumage and will be influenced mainly by air temperature. Other areas, with little or no insulative pelage or cover, will appear as "thermal windows" representing skin temperature. Simply, in a relatively non-hairy animal such as man or some pigs surface temperature in a thermogram will reflect primarily skin temperature, whereas, in a heavy or dense coated animal e.g. a fully fleeced sheep or densely feathered bird the thermogram will indicate the surface temperature of the insulating cover over much of the body with the exception of the less well covered regions such as the face, eye, parts of the legs and feet. In order to interpret the observed surface temperatures of animals it is important to consider the basic principles of thermoregulation.

Thermoregulation

Homeothermic animals are capable of regulating deep body temperature within a narrow range through a number of behavioural, metabolic and physiological processes. Behavioural strategies may involve relocation, seeking cover or shade, changes in posture and configuration or altered activity and feeding (Monteith 1973, Monteith and Mount 1974; Yousef 1985). The physiological and metabolic responses include adjustments in metabolic heat production and heat loss through altered insulation and tissue heat transfer. Thus, as the thermal microenvironment changes the animal may firstly adjust its rate of heat loss to maintain a constant body temperature and under more extreme conditions may then recruit adaptations in heat production. The animal may be generally regarded as in a thermoneutral condition when the body temperature is maintained at the required constant level by regulatory processes other than altered metabolic rate. The boundaries at which changes in metabolic heat production are induced are defined by the upper and lower critical temperatures. The relationships between metabolic heat production, deep body temperature and environmental temperature are presented diagrammatically in Figure 1.

Within the thermo-neutral zone (as defined by Yousef 1985) the body temperature regulation is achieved by mechanisms other than changes in metabolic rate or increased evaporative heat loss. These are depicted diagrammatically in Figure 2.

Therefore, it is clear that animals will control metabolic heat production and loss in accordance with the demands imposed by the external thermal environment. Regardless of any adjustments in metabolic rate the regulation of heat loss will involve physiological homeostatic mechanisms that will increase or decrease transfer of heat from the core of the animal to the periphery. This may be achieved by altering blood flow from the body core to the heat exchange surfaces e.g. the skin and/or respiratory tract and by changing the insulation of the pelage or feather cover (ptilo or ptero-erection). Vasodilation or vasoconstriction in the periphery, and in particular the skin, thus regulates delivery of heat to the exchange surfaces and the effective insulation. The insulative properties of any overlying hair, coat or feather cover will then alter heat exchange between skin and the surrounding air. Erection of the hair or feathers controls the insulation efficacy of the pelage. In physical terms the animal may regarded theoretically as consisting of a number of thermal shells. It may be proposed that the body can be considered as a series of concentric thermal shells (Mitchell unpublished). The body core is surrounded by concentric shells at different temperatures with a gradient from core to periphery (Figure 3). The transfer of heat between shells is controlled by the physiological regulatory mechanisms. The outermost shell may be regarded as the skin or pelage. As environmental temperature increases, the requirement for increased heat loss results in greater heat transfer from the inner to outer shells.

The outermost shells will increase in temperature and the gradient from core to periphery will decrease (Figure 4). In this situation the surface temperature will be closer to the actual core temperature. This will be more apparent in body surface areas where additional insulation in the form of hair or feathers is minimal. In cold conditions the surface or skin of the animal will have reduced blood flow in order to conserve heat by reducing loss and in warmer conditions increased heat transfer from core to periphery will result in higher temperatures at the heat exchange surfaces. From the perspective of thermal imaging these are important factors. The thermograms of animals in different thermal environments will reflect the adaptive thermoregulatory mechanisms and the control of heat transfer from core to periphery. In warm environments the surface temperatures of the heavily insulated areas of the body will increase less than those of the less well insulated or skin areas. This may not reflect the concurrent changes in skin (underlying) temperatures but is a function the limited rate of heat transfer from the skin to the surface of a thick coat. In many animals specific body areas that have minimal pelage or insulation may operate as high exchange thermal windows during heat stress and these may be clearly identified in thermograms. It is thus proposed that thermal imaging will yield thermal maps of the surface of the outermost thermal shell of an animal and that the measured values will be a product of heat transfer from the core of the animal, the degree of insulation offered by any coat or feather cover and the ambient temperature and other environmental variables such as air movement and water vapour.

Heat exchange in the pelage/ plumage. Surface and skin temperature

The coats and plumages of animals and birds are regarded as the major component of external insulation (Clark et al 1973; Campbell 1977; Wolf and Walsberg 2000; Turnpenny et a 2001a) and the main determinant of heat loss may be the coat thickness. The effectiveness of the insulation may be altered by changes in the degree of hair or feather erection thus effectively changing the thickness or by external factors such as wetting and air movement (Campbell et al 1980; McArthur and Ousey 1994, 1996; Gebremedhin and Wu 2002). The pelage or plumage thus constitutes a dynamic barrier to heat transfer but heat exchange within the hair, fur or feather is complex also involving radiative loss and gain, conduction and convection and potential storage of latent heat (Clark et al 1973; Cena and Monteith 1975 a and b; Campbell 1977; McArthur 1981, 1991). The thermal transfer processes in animal coats were difficult to determine accurately until the use of thermography facilitated precise measurement of the temperature at the heat exchange surface of the coat simultaneous with measurement of skin and air temperatures (Clark et al 1973). These early studies established the value of thermography in studies on heat exchange in animals and through their pelage or plumage and lead to the promotion of the technique in animal science and veterinary medicine (Cena and Clark 1973 a and b; 1977). The insight provided by this work later facilitated the incorporation of more accurate assessments of the influence of coats and feather cover on thermal exchange into integrative, comprehensive models (e.g. Walsberg et al 1978; McArthur 1980; McGovern and Bruce 2000; Turnpenny et al 2000 a and b). The insulative role and capacity of thick pelage is illustrated in the contract between the facial and fleece surface temperatures of a sheep in Figure 5 (SRUC 2012 ©).

Thermographic analysis of body surface temperature and its regulation in mammals have been extensively reviewed and modelled (Phillips and Heath 1995; Mortola 2013). The temperature of exposed or naked skin is affected by the deep body temperature of the subject, the environmental temperature, the degree of wetting of the surface and the local water vapour density. In the case of outdoor animals, any incident log wave radiation will influence heat balance along with the rate of heat transfer from the core of the animal to the periphery. The latter will be controlled by the degree or balance of vasoconstriction-vasodilatation in the skin circulation and that of immediate sub-cutaneous structures (e.g. muscles). Thus, if thermograms of skin are analysed the interpretation of the data must take in to account the thermal microenvironment of the animal and any factors that might influence the general or local metabolic heat production of the animals, the peripheral blood flow and in particular the skin micro-circulation. In most animals, thermal imaging will involve composite analysis involving some naked skin areas, some specific surface structures such as the eye, ear, feet or wings and more insulated areas covered by hair, fur or feathers. The thermal images of each area may be employed with a number of different scientific objectives in mind. Some studies may address the overall heat exchange or body temperature of the animals whereas other approaches may involve examination of thermal exchange in the coat, from skin or through thermal windows.

Thermal imaging – physiological applications

Thermal imaging represents a useful adjunct to other measures in animal science including physiological studies. The technique allows non-contact monitoring of important physical variables in live and conscious animals and is minimally invasive and does not interfere with normal behaviours (Stewart et al 2005; Knizkova et al 2007; Vadivambal and Jayas 2011) A wide range of animal science applications and future directions, ranging from thermal physiology e.g. small area, specific surface site heat exchange to large scale animal counting, have been described by McCafferty (2010).The general principles and applications of infra-red thermography in relation to heat exchange in physiological studies in animals have been reviewed by Speakman and Ward (1998). Further applications of thermography in veterinary medicine including assessment of physiological status through surface or skin temperature measurements have been reviewed by Stelletta et al (2012 - also see elsewhere in this publication). Thus, surface or skin temperatures may be measured and characterised by thermal imaging or thermography for a number of physiological purposes either alone or in conjunction with other measures.

Measurements of body temperature

It has been proposed that "advances in thermal imaging technology" have shed light, importantly, upon the regulation of peripheral blood flow in endothermic animals, on the dynamics of animal heat transfer in complex thermal environments on the production of heat associated with metabolism and on the importance of evaporative heat loss to respiratory function and its potential contributions to prevent overheating of the brain (Tattersall and Cadena 2010). In man, thermal imaging is proposed as an accurate and reliable method for estimating deep body temperature and the detection of hyperthermia or pyrexia (Cheung et al 2008, 2012). In the "relatively non-hairy" human a number of skin sites might be employed but the inner canthus of the eye is considered to produce the most reliable results (Haddadin et al 2005; Teunissen et al 2011). The accuracy of estimation deep body temperature from surface measures is assessed by correlation with rectal or other accepted measures of core temperatures. Using this approach eye temperature has been employed to estimate deep body temperature in sheep (Willard et al 2006) and cattle (Abreu et al 2010). Figure 6 (SRUC 2012 ©) shows how the eye temperature may be measured in a cow by high resolution thermal imaging in the lateral configuration and Figure 7 (SRUC 2012 ©) illustrates the frontal determination and head and facial temperatures in cows in body temperature studies. Studies in mole rat revealed strong correlations between core temperature and eye and peripalpebral temperature (Sumbera et al 2007). Useful correlations between eye temperature and rectal temperature have been reported in ponies by Johnson et al (2011) who proposed the use of thermal imaging to detect febrile states in these animals. In pigs, early studies disputed the use of surface temperature measurements to detect elevated body temperature (Wendt et al 1997; Loughmillar et al 1999; DeWulf et al 2003) but more recent reports suggest that selection of appropriate body surface areas (e.g. vulva and teats) in sows will yield reliable predictions of core temperature (Traulsen et al 2010). Warriss et al (2006) have previously claimed a good correlation ($r = 0.71$, $p<0.001$) between ear temperature and blood (core) temperature at exsanguination. Facial temperature in broiler chickens under commercial conditions exhibits a good correlation with deep body temperature according to Giloh et al (2012) and may be used to detect acute heat stress in all ages of birds studied from 8-36 days. Yahav and Giloh (2012) confirmed these finding and asserted that thermography might be usefully applied to determining body temperature, from facial temperature in the detection of heat stress in commercial flocks of broiler chickens. Figure 8 (SRUC 2012 ©) shows the facial temperature of a young turkey under cool conditions and illustrates how selection of the appropriate area for body temperature estimation can be made using a high resolution thermal imaging camera. Using other areas of the body for surface temperature measurement, particularly in animals with a coat, is less likely to yield accurate estimates of deep body temperature e.g. Das et al (1997) using buffalo found no usable relationships between surface temperature and core temperature. Mitchell 2013 have examined the use of surface temperature measurements in pigs to estimate deep body temperature and to assess the degree of heat stress experienced at elevated ambient temperatures. The range of environmental temperatures employed was -5 to +35°C. Correlation of skin temperatures with deep body temperatures in pigs indicated that no single site or mean skin temperature could be usefully employed to predict deep body temperature when the whole range of environmental conditions, from cold to hot, were considered. However, it was proposed that surface temperature measurement alone may be usefully employed specifically in conditions likely to induce heat stress i.e. above the thermoneutral range of environmental temperatures or heat loads. It was stated that when core temperature is increasing the surface temperature will increase to facilitate heat loss (increased skin perfusion). If core temperature continues to rise, the skin temperature increases until it approaches core temperature. It is therefore postulated that very high mean skin temperatures (at elevated ambient temperatures) in non-febrile pigs may be a useful index of the degree of heat stress. The estimation of mean surface temperature or maximum regional temperature by means of thermal imaging and appropriate image analysis may provide a sound basis for such an approach and also for the assessment of the degree of heat stress imposed during exposure to high ambient temperatures. In order to utilise thermal images to assess deep body temperature in this way and to develop the necessary models it is essential to identify the appropriate surface sites for each species and to develop the appropriate predictive models over as wide a range of deep body temperatures as possible.

Heat Exchange

Thermal imaging and infra-red thermometry have an important role in the estimation of heat exchange between animals and their environment. The models employed require accurate measurement or calculation of mean or regional radiative temperatures of the animals' surface. The methods for calculation of mean surface temperatures derived thermographically and comparisons with other approaches have been reviewed (Choi et al 1997). From surface temperature values and measures of air temperature and environmental radiant temperature it is possible to calculate total sensible heat loss. Many studies have developed models to estimate heat exchange and thermal balance for different species in this way (Turnpenny et al 2000 a and b); Fiahlo et al 2004; Huynh et al 2005). Thus, radiant and convective heat exchange may be determined from the appropriate temperature gradients and fundamental physical principles. This approach has been employed in studies on heat loss from a number of species including ratites (Phillips and Sanborn 1994), horses (Morgan et al 1997; Autio et al 2006, 2007), barn owls (McCafferty et al 1998), pigs (Loughmillar et al 2000; Huynh et al 2005), cattle (Keren and Olson 2006), turkeys (Yahav et al 2011), and penguins and seals (McCafferty et al 2011 and Paterson et al 2012). In addition to estimation of total heat loss, regional heat loss determined from thermographic analysis may be related to physiological function and adaptation to thermal challenge as reported in studies on woodchuck (Phillips and Heath 2001) and otters (Kuhn and Meyer 2009) and in analysis of the significance of "thermal windows" in adaptive heat loss as in vultures (Ward et al 2008), elephants (Weissenbock et al 2010), camels (Abdoun et al 2012) and harbour seals (Nienaber et al 2010; Erdsack et al 2012). A number of studies have utilised thermography to characterise the surface temperature profiles and distributions in the domestic fowl (Tessier et al 2003; Naas et al 2010) and to model radiant and convective heat loss from the birds (Yahav and Giloh 2012). Models that facilitate prediction of heat loss may also underpin estimation of metabolic energy use and requirements and changes therein resulting from environmental conditions (e.g. Keren and Olsen 2007; Yahav et al 2011; Yahav and Giloh 2012; Case et al 2012). The extensive analysis and quantification of surface temperatures and heat exchange through thermography facilitates determination of important thermal characteristics of animals including insulative properties of the pelage (e.g. Kotrba 2007; Gerken 2009) and determination of critical temperatures (e.g. Autio et al 2007) and the thermoneutral zone (Romanovsky et al 2002). When measuring surface temperatures in coated animals in it is important to examine any additional factors that might influence the analysis e.g. coat wetting. Figure 9 (SRUC 2013 ©) shows patches of apparently reduced temperature on the coat of a cow which resulted from the animal licking those areas.

Physiological status

Analysis of surface of skin temperature using thermography may allow characterisation of changing physiological status. Thus, increased local metabolism in voles e.g. in brown adipose tissue in response to a thermal challenge may be assessed by thermography of the overlying skin areas (Jackson et al 2001). The relationship between thermoregulatory capacity and fertility has been studied in male llamas (Schwalm et al 2008) using thermography and physiological measures. Declining fertility associated with heat stress was demonstrated and the reliance of the llama on effective heat loss through surface "thermal windows" (vide infra) was established. GnRH administration and increased testosteronemia in alpacas are associated with an increased scrotal temperature that may be quantified by thermography (Stelletta et al 2012). The same authors describe the use of thermal imaging to monitor oestrus and ovulation in mares (horses). It has been reported that thermographic analysis of surface temperature may be used to detect oestrus in gilts (pigs) (Sykes et al 2012) and to confirm mid to late gestation in mares (horses) (Bowers et al 2009). In camels lactation is associated with increases in body, udder and teat temperature examined by thermal imaging (Samara et al 2013). Thermal imaging may also be employed as a non-invasive method for quantifying torpor in small mammals (Warnecke 2012). Yahav and Giloh (2012) have described the development and application of models to determine radiant and convective heat loss from poultry on the basis of surface temperature determined by thermography. The studies report the application of the models and methods to assessment of vasomotor responses in thermally conditioned birds and correlations between surface temperatures and endocrine (thyroid hormone and vasotocin) responses to heat stress. Physiological responses of pigs to heat stress have been shown to correlate well with the outputs of models of thermal exchange based on surface temperature measurements in pigs (Huynh et al 2005). Physiological stress status and responses to acute and chronic stressors may be assessed effectively by infra-red thermography or IRT (Stewart et al 2005). It is suggested that appropriate application of IRT can allow measurement of different components of the stress axis including acute sympathetic and hypothalamic-pituitary-adrenocortical responses.

Micro-circulatory changes

Local or regional changes in blood flow resulting from vasomotor changes in response to physiological stimuli, pathology or environmental challenge may be characterised by means of thermography Sagaidachnyi et al 2012). Neural damage and dysfunction or local anaesthesia will also alter regional blood flow and the effects can be quantified by thermography (Holmes et al 2003; Ruijs et al 2011; Lange et al 2011). Figure 10 shows blood flow to the ear of an anaesthetised pig in a study comparing the effects of different pre-surgical sedation regimes on vasomotor tone (SRUC 2013 ©)

Muscle activity

Increased muscle activity and metabolism will result in increased heat transfer to the overlying tissues and skin and may be detected as increased surface temperature. Thermal imaging may thus be employed to characterise differential muscle use (Vainionpaa et al 2012) and the development of fatigue during exercise (Bartuzi et al 2012).

Methane production

In dairy cattle it has been demonstrated (Montanholi et al 2008) that thermography can allow prediction of metabolic heat loss from the animals and that a regional difference in surface temperature (left to right flank) correlates significantly ($y = 113x + 396$; $R^2 = 0.59 - p<0.01$) with methane production. These findings could constitute an important new non-invasive methodology for the measurement of methane emissions form ruminants.

Summary

There are a large number of possible applications of thermography or thermal imaging to the study of human and animal physiology. The examples provided in this chapter constitute only a small proportion of the potential catalogue of physiological systems and responses that might be examined by thermography. The most obvious areas of application are determination of body temperature, regional temperature and heat exchange, characterisation of physiological status and stress, identification of altered metabolic state and muscle activity and changes in vasomotor tone and microcirculation in the skin. Any physiological or metabolic system or response that will influence heat production, transfer and loss may cause changes in delivery of heat to the animal surface. Such changes can be detected by thermal imaging and quantified and these measures related to the underlying mechanisms and processes. The practical and theoretical considerations, constraints and limitations described in the current chapter and elsewhere in this publication must inform those intending to employ successfully the methodology in physiological studies.

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REFERENCES

1) Abdoun, K.A., Samara, E.M. Okab, A.B. and Al-Haidary A.A. (2012) Regional and circadian variations of sweating and body surface temperature in camels (Camelus domedarius). Animal Science Journal, 83, 556-561.
2) Abreu, M.M,. Haddadin, S.S. , Hott, M., Assis, A., Silverman, D.G. (2010) Consistency of Brain Temperature Tunnel Measurements in Different Environmental Temperature. From Proceedings of the 2010 Annual Meeting of the American Society Anesthesiologists, October 17th 2010 Anesthesiology: 113: A673
3) Autio, E. Neste, R., Airaksinen, S and Heiskanen, M-L. (2006) Measuring the heat loss in horses in different seasons by infrared thermography. Journal of Applied Animal Welfare Sceince, 9:211-221.
4) Autio, E. Heiskanen, M-L., and Mononen, J. (2007) Thermographic evaluation of the lower critical temperature in weanling horses. Journal of Applied Animal Welfare Science, 10: 207-216.
5) Bartuzi, P., Roman-Liu, D. AND Wisniewski, T. (2012) The influence of fatigue onmuscle temperature. International Journal of Occupational Safety and Ergonomics, 18(2): 233-243.
6) Bowers, S., Gandy, S., Anderson, B., Ryan, P. and Willard, S. (2009) Assessment of pregnancy in the late-gestation mare using digital infrared thermography. Theriogenology, 72: 372-377.

7) Campbell, G.S.(1977) An introduction to environmental physics. Heidelberg Science Library, Publisher Springer-Verlag, New York, ISBN 0 387 90228-7. 159pp.
8) Campbell, G.S., McArthur, A.J. and Monteith, J.L. (1980) Wind speed dependence of heat and mass transfer through coats and clothing. Boundary Layer Meteorology 18: (4) 485-493.
9) Case, L.A., Wood, B.J. and Miller, S.P. (2012) Investigation of body surface temperature measured with infrared imaging and its correlation with feed efficiency in the turkey (Meleagris galopavo). Journal of Thermal Biology, 37: 397-401.
10) Cena, K., Clark, J.A. (1973a) Thermographic measurements of the surface temperaturas of animals. Journal of Mammals, 54(4): 1003-1007.
11) Cena, K., Clark, J.A. (1973b) Thermal radiation from animal coats: coat structure and measurements of radiative temperature. Physics, Medicine and Biology, 18(3): 432-443.
12) Cena, K. and Clark, J.A. (1974) Heat balance and thermal resistances of sheep's fleece. Phys. Med. Biol. 19, 51-65.
13) Cena, K. and Monteith, J.L. (1975) Transfer processes in animal coats II. Radiation. Proceedings of the Royal Society of London, Series B 188: 377-393
14) Cena, K. and Monteith, J.L. (1975) Transfer processes in animal coats. II. Conduction and convection. Proceedings of the Royal Society of London, Series B 188: 395-411
15) Cheung, B.M.Y., Chan, L.S., Lauder, I.J. and Kumana, C. (2008) Detection of human body temperature with infrared thermographic imaging. Accuracy and feasibility in detection of fever in human subjects. Final Report RFCID # 03040232
16) Cheung, B.M.Y., Chan, L.S., Lauder, I.J. and Kumana, C. (2012) Detection of body temperature with infrared thermography. Accuracy in detection of fever. Hong Kong Medical Journal, 18 (suppl 3) S31-34.
17) Chui, J.K., Miki, K., Sagawa, S. and Shiraki, K. (1997) Evaluation of mean skin temperature formulas by infrared thermography. International Journal of Biometeorology, 41:68-75.
18) Clark, J.A., Cena, K. and Monteith, J.L. (1973) Measurements of the local heat balance of animal coats and human clothing. Journal of Applied Biology, 35 (5): 751-754.
19) Clark, J.A. and Cena, K. (1977) The potential of infra-red thermography in veterinary diagnosis. Veterinary Record 100(19): 402-404.
20) Das, S.K. Upadhyay, R.C. and Madan, M.L. (1997) Surface and core temperature changes in Murrah buffaloes during cold weather conditions. Indian Journal of Animal Sciences, 67: 581-584.
21) Dewulf, J., Koenen, F., Laevens, H. and de Kruif, A. (2003) Infrared thermometry is not suitable for the detection of fever in pigs. Vlaams Diergeneeskundig Tijdschrift, 72: 373-379.
22) Erdsack, N., Hanke, F.D., Denhardt, G. and Hanke, W. (2012) Control and amountof heat dissipation through thermal windows in the harbour seals (Phoca vitulina). Journal of Thermal Biology, 37:537-544.
23) Fialho, F.B., Bucklin, R.A., Zazueta, F.S. and Myers, R.O. 2004a. Theoretical model of heat balance in pigs. Animal Science, 79, 121-134.
24) Gebremedhin, K.G. and Wu, B. (2002) Simulation of sensible and latent heat losses form wet-skin surface and fur layer. Journal of Thermal Biology, 27: 291-297.
25) Gerken, M. (2010) Relationships between integumental characteristics and thermoregulation in South American camelids. Animal, 4(9): 1451-1459.
26) Giloh, M., Shinder, D. and Yahav, S. (2012) Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. Poultry Science, 91:175-188.

27) Haddadin, S.S. , Abreu, M.M, Silverman, D.G., Luther, M. and Hines, R.L. (2005) Non-invasive assessment of intracranial temperature via the medial canthal-brain temperature tunnel. From Proceedings of the 2005 Annual Meeting of the American Society Anesthesiologists, October 22nd 2005 Anesthesiology 103: A38
28) Holmes, L.C., Gaughan, E.M., Gorondy, D.A., Hogge, S. and Spire, M.F. (2003) The effect of perineural anaesthesia on infrared thermographic images of the forelimb digits of normal horses. Canadian Veterinary Journal, 44: 392-396.
29) Huynh, T.T.T., Aarnink, A.J.A., Verstegen, M.W.A., Gerrits, W.J.J., Heetkamp, M.J.W., Kemp, B., Canh, T.T., 2005c. Effects of increasing temperature on physiological changes in pigs at different relative humidities. J. Anim. Sci. 83, 1385-1396.
30) Jackson, D.M., Hambly, C., Trayhurn, P and Speakman, J.R. (2001) Can non-shivering thermogenesis in brown adipose tissue following NA injection be quantified by changes in overlying surface temperatures using infrared thermography? Journal of Thermal Biology, 26:85-93.
31) Johnson, S.R., Rao, S., Hussey, S.B., Morley, P.S. and Dargatz, J. (20110) Thermographic eye temperature as an index to body temperature in ponies. Journal of Equine Veterinary Science, 31(2): 63-66.
32) Kastberger, G. and Stachl, R. (2003) Infrared imaging technology and biological applications. Behavior Research Methods, Instruments and Computers, 35: 429-439.
33) Keren, E.N. and Olson, B.E. (2006) Thermal balance of cattle grazing winter range: Model development. Journal of Thermal Biology, 31:371-377.
34) Keren, E.N. and Olson, B.E. (2007) Applying thermal imaging software to cattle grazing winter range. Journal of Thermal Biology, 32:204-211.
35) Knizkova, I., Kunc, P., Gurdil, G.A.K., Pinar, Y. and Selvi, K.C. (2007) Applications of infrared thermography in animal production. Journal of the Faculty of Agriculture OMU, 22:329-336.
36) Kotrba, R., Knizkova, I., Kunc, P. and Bartos, L. (2007) Comparison between the coat temperature of the eland and dairy cattle by infra-red thermography. Journal of Thermla Biology, 32: 355-359.
37) Kuhn, R.A. and Meyer, W. (2009) Infrared thermography of the body surface in the Eurasian otter *Lutra lutra* and the giant otter *Pteronura brasiliensis.* Aquatic Biology, 6: 143-152.
38) Lange, K.H.W., Jansen, T., Asghar, S., Kristensen, P.L., Skonnemand, M. and Norgaard, P. (2011) Skin temperature measured by infrared thermography after specifc ultrasound-guided blocking of the musculo-cutaneous, radial, ulnar and medina nerves in the upper extremity. British Journal of Anaesthesia, advance publishing doi:10.1093/bja/aer085. 9 pages.
39) Loughmiller, J.A., Spire, M.F., Dritz, S.S., Fenwick, B.W., Hogge, S.B. and Foster, J. (1999) Infrared thermography of swine body temperature and associated rectal temperatures during an acute respiratory disease challenge. Food Animal Health and Management Centre, Swine Day 1999 proceedings 33-36.
40) Loughmiller, J.A., Spire, M.F., Tokach, M.D., Dritz, S.S., Nelssen, J.L., Goodband, R.D., Hogge, S.B. and James, B.W. (2000) Use of infrared thermography to evaluate differences in mean body surface temperature and radiant heat loss in growing pigs. Food Animal Health and Management Centre, Swine Day 2000 proceedings pp3.
41) McArthur, A.J., 1981. Thermal resistance and sensible heat loss from animals. J. Thermal Biol. 6, 43-47.
42) McArthur, A.J., 1987. Thermal interaction between animal and microclimate: A comprehensive model. J. Theor. Biol. 126, 203-238.
43) McArthur, A.J., 1990. Thermal interaction between animal and microclimate: Specification of a “standard environmental temperature” for animals outdoors. J. Theor. Biol. 148, 331-343.

44) McArthur, A.J., 1991. Thermal radiation exchange, convection and the storage of latent heat in animal coats. Agr. Forest. Meteorol. 53, 325-336.
45) McArthur, A.J. and Ousey, J.C. (1994) Heat loss form a wet animal: changes with time in the heat balance of a physical model representing a new born homeotherms. Journal of Thermal Biology, 19(2): 81-89.
46) McArthur, A.J. and Ousey, J.C. (1996) The effect of moisture on the thermal insulation provided by the coat of a thoroughbred foal. Journal of Thermal Biology, 21(1): 43-48.
47) McCafferty, D.J. (2007) The value of infrared thermography for research on mammals: previous applications and future directions. Mammal Review, 37:207-223.
48) McCafferty, D.J., Moncreiff, J.B., Taylor, I.R. and Boddie, G.F. (1998) The use of IR thermography to measure the radiative temperature and heat loss of a barn owl (Tyto alba) Journal of Thermal Biology, 23:311-318.
49) McCafferty, D.J., Gilbert, C. Paterson, Pomeroy, P.P., Thompson, D., Currie, J.I and Ancel, A. (2011) Estimating metabolic heat loss in birds and mammals by combining infrared thermography with biophysical modelling. Comp. Biochem. Physiol. A, 158: 337-345.
50) McGovern, R.E. and Bruce, J.M. (2000) A model od the thermal balance for cattle in hot conditions. Journal of Agricultural Engineering Research, 77:81-92.
51) Mitchell, M.A. (2013) Measurement of body temperature in animal studies: how, when, where and why? Proceedings of the EAAP Annual Meeting, Nantes, France, August 2013 – in the press.
52) Montanhioli, Y.R., Odongo, E., Swanson, K.C. and Schenkel, F.S. (2008) Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature response to physiological events in dairy cattle (Bos taurus). Journal of Thermal Biology, 33:468-475.
53) Monteith, J.L. (1973) Principles of Environmental Physics. Contemporary Biology, Publisher Edward Arnold UK, ISBN 0 7131 2375 3 - 241pp.
54) Monteith, J.L. and Mount, L.E. (1974) Heat Loss form Animal and Man: Assessment and Control, Butterworth, London, 1974, 457pp
55) Morgan, K., Ehrlemark, A. and Sallvik, K. (1997) Dissipation of heat from standing horses exposed to ambient temperatures between -3°C and 37°C. Journal of Thermal Biology, 22:177-186.
56) Mortola, J.P. (2013) Thermographic analysis of body surface temperature of mammals. Zoological Science, 30(2): 118-124.
57) Mount, L.E., 1979. Adaptation to the Thermal Environment. Contemporary Biology, Publisher Edward Arnold, UK. 333pp
58) Naas, I de A., Romanini, C.E.B., Neves, D.P., do Nascimento, G.R. and Vercellino, R do A. (2010) Broiler surface temperature distribution of 42 day old chickens. Sci. Agic. (Brazil), 67:497-502.
59) Nanni-Costa, L., Redaelli, V., Magnani, D., Cafazzo,S., Amadori, M., Razzuoli,E., Verga, M. and Luzi, F. (2011) Relationship between piglets skin temperature measured by infrared thermography and environmental temperature in a vehicle in transit: a preliminary study. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 112, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7
60) Nienaber, J., Thonton, J., Horning, M., Polasek, L. and Mellish, J-A. (2010) Surface temperature patterns in seals and sea lions: A validation of temporal and spatial consistency. Journal of Thermal Biology, 35:435-440.

61) Paterson, W., Sparling, C.E., Thompson, D., Pomeroy, P.P., Currie, J.I. and McCafferty, D. (2012) Seals like it hot:Changes in surface tempertuare of harbour seals (Phoca vitulina) form late pregnancy to moult. Journal of Thermal Biology, 37:454-461.
62) Phillips, P.K. and Sanborn, A. (1994) An infra-red, thermographic study of surface temperature in three ratites: ostrich, emu and double-wattled cassowary. Journal of Thermal Biology, 19:423-430.
63) Phillips, P.K. and Heath, J.E. (1995) Dependency of surface temperature regulation on body size in terrestrial mammals. Journal of Thermal Biology, 20: 281-289.
64) Phillips, P.K. and Heath, J.E. (2001) An infra-red thermographic study of the surface temperature in the euthermic woodchuck (Marmota monax). Comp. Biochem. Physiol. A, 129:557-562.
65) Redaelli, V., Farish, M., Luzi, F. and Baxter, E. (2011) Demonstration of thermoregulatory control of piglets during farrowing by infrared thermography. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 88, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7
66) Redaelli, V., Luzi, F. Verga, M. and Farish, M. (2011) Thermographic techniques to assess welfare during teeth grinding in piglets. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 87, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
67) Romanovsy, A., Ivanov, A.I. and Shimansky, Y.P. (2002) Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. Journal of Applied Physiology, 92: 2667-2679.
68) Ruijs, A.C.J., Niehof, S.P., Hovius, S.E.R. and Selles, R.W. (2011) Cold induced vasodilatation following traumatic median or ulnar nerve injury. Journal of Hand Surgery 2011 – doi:10.1016/j.jhsa.2011.02.009
69) Sagaidachnyi, A. A., Usanov, D. A., Skripal, A.V. and Fomiin, A.V. (2012) Correlation of skin temperatures and blood flow oscillations. Proceedings of the SPIE (Optical Technologies in Biohysics and Medicine XIII – SARATOV Fall Meeting 2011) – Volume 8337.
70) Schwalm, A., Erhardt, G., Gerken, M., Moors, E., Gauly, M. (2008) Influence of heat stress on thermoregulation in shorn and unshorn male llamas. Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere, 36: 324-328.
71) Speakman, J.R. and WARD, s. (1998) Infrared thermography: Principles and Applications. Zoology, 101: 224-232.
72) Stelletta,C., Gianesella, M., Vencato, J., Fiore, E. and Morgante, M. (2012). Thermographic Applications in Veterinary Medicine, Infrared Thermography, Dr. Raghu V Prakash (Ed.), ISBN: 978-953-51-0242-7, InTech, Available from: http://www.intechopen.com/books/infraredthermography/ thermographic-applications-in-veterinary-medicine.
73) Stewart, M., Webster, J.R., Schaefer, A.L., Cook, N.J. and Scott, S.L. (2005) Infrared thermography as a non-invasive tool to study animal welfare. Animal Welfare, 14:319-325.
74) Sumbera, R., Zelova, J., Kunc, P., Knizkova, I. and Burda, H. (2007) Patterns of surface temperatures in two mole-rats (Bathyergidae) with different social systems as revealed by IR-Thermography. Physiology and Behaviour, 92:526-532.
75) Sykes, D.J., Couvillion, J.S., Cromiak, A., Bowers, S., Schenk, E., Crenshaw, M. and Ryan, P.L. (2012) The use of digital infrared thermal imaging to detect estrus in gilts. Theriogenology, 78: 147-152.

76) Tattersall, G.J. and Cadena, V. (2010) Insights in to animal temperature adaptations revealed through thermal imaging. Imaging Science Journal, 58: 261-268.
77) Tessier,M., Du Tremblay, D., Klopfenstein, C., Beauchamp, G. and Boulianne, M. (2003) Abdominal skin temperature variation in healthy broiler chickens as determined by thermography. British Poultry Science 82:846-849.
78) Teunissen, L.P.J. and Daanen, H.A.M.(2011) Infrared thermal imaging of the inner canthus of the eye as an estimator of body core temperature. Journal of Medical Engineering and Technology, 35: 134-138.
79) Traulsen, I., Naunin, K., Muller, K. and Krieter, J. (2010) Application of infrared thermography to measure body temperature of sows. Zuchtungskunde, 82: 437-446.
80) Turnpenny, J.R., McArthur, A.J., Clark, J.A., Wathes, C.M., 2000. Thermal balance of livestock. 1. A parsimonious model. Agr. Forest Meteorol. 101, 15-27.
81) Turnpenny, J.R., Wathes, C.M., Clark, J.A., McArthur, A.J., 2000. Thermal balance of livestock. 2. Applications of a parsimonious model. Agr. Forest Meteorol. 101, 29-52.
82) Vadivambal, R. and Jayas, D.S. (2011) Applications of thermal imaging in agriculture and food industry – A review. Food Bioprocessing Technology, 4:186-199.
83) Vainionpaa, M., Tienhaara, E-P., Raekallio, M., Junnila, J., Snellman, M. and Vainio, O. (2012) Thermographic imging of the superficial temperature in racing greyhounds befor and after the race. The Scientific World Journal, 2012, 6 pages article ID 182749. Doi:10.1100/2102/182749
84) Valle, E, Redaelli, V., Papa,I., Bergero, D and Luzi, F. (2011) Infrared thermography: a non-invasive technique to assess metabolic activity in horses. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 113 Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
85) Walsberg, G.E., Campbell, G.S. and King, J.R. (1978) Animal coat color and radiative heat gain: a re-evaluation. Journal of Comparative Physiology, 126: 211-222.
86) Ward, J., McCafferty, D.J., Houston, D.C. and Ruxton, G.D. (2008) Why do vultures have bald heads? The role of postural adjustment and bare skin areas in thermoregulation. Journal of Thermal Biology, 33:168-173.
87) Warnecke, L. (2012) Quantifying torpor in small mammals non-invasively using infrared thermocouples. Journal of Thermal Biology, 37:380-383.
88) Warriss, P.D., Brown, S.N., Knowles, T.G., Wilkins, L.J., Pope, S.J., Chadd, S.A., Kettlewell, P.J., Green, N.R., 2006a. Comparison of the effect of fan-assisted and natural ventilation of vehicles on the welfare of pigs being transported to slaughter. Vet. Rec. 158, 585-588.
89) Warriss, P.D., Pope, S.J., Brown, S.N., Wilkins, L.J., Knowles, T.G., 2006b. Estimating the body temperature of groups of pigs by thermal imaging. Vet. Rec. 158, 331-334.
90) Warriss, P.D., Pope, S.J., Brown, S.N., Wilkins, L.J., Knowles, T.G., 2006b. Estimating the body temperature of groups of pigs by thermal imaging. Vet. Rec. 158, 331-334.
91) Weissenbock, N.M., Weiss, C.M., Schwammer, H.M. and Kratochvil, H. (2010) Thermal windows on the body surface of African elephants (Loxodonta Africana) studied by infrared thermography. Journal of Thermal Biology, 35:182-188.
92) Wendt, M., Eickhoff, K. and Koch, R. (1997) Measuirng of the skin tempertuare as a method to detect pigs with elevated body temperature. Deutsche Tierarztliche Wochenschrift, 104: 29-33.
93) Willard, S.T., Vinson, M.C. and Godfrey, R.W. (2006) Digital infrared thermal imaging of the eye as correlated to rectal and vaginal temperature measurements in the ewe. Journal of Animal Science, 84: 434

94) Wolf, B.O. and Walsberg, G.E. (2000) The role of plumage in heat transfer processes in birds. American Zoology, 40:575-584.
95) Yahav, S., Druyan, S., Rusal, M. and Shinder, D. (2011) Diurnally cycling temperature and ventilation affect young turkeys' performance and sensible heat loss. Journal of Thermal Biology, 36: 334-339.
96) Yahav,S and Giloh, M. (2012). Infrared Thermography - Applications in Poultry Biological Research, Infrared Thermography, Dr. Raghu V Prakash (Ed.), ISBN: 978-953-51-0242-7, InTech, Available from: http://www.intechopen.com/books/infrared-thermography/infrared-thermography-applications-in-agricultureand- biological-research
97) Yousef, M.K., 1985. Stress physiology in livestock. CRC Press, Boca Raton, FL. 217pp.

Figure 1 – The upper (UCT) and lower critical temperatures (LCT) are indicated by points c and d. The thermo-neutral zone extends between these two points. Below the LCT animals must increase their metabolic rate to maintain deep body temperature (b-c) and above the UCT must increase evaporative heat loss. At points a and e homeostatic control becomes inadequate and body temperature falls or rises.

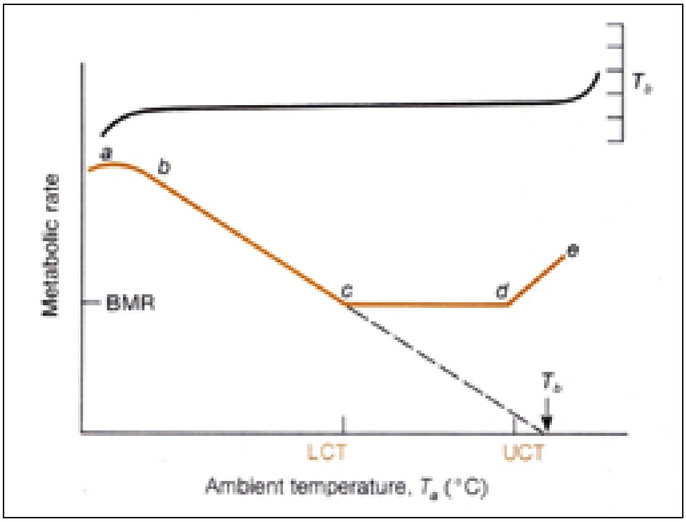


Figure 2 – Physiological effector intensity and temperature. Over a narrow range core temperature may be controlled by pilomotor responses and altered peripheral insulation through vasomotor tone (between UCT and LCT). Outwith this range metabolic heat production or evaporative heat loss must increase.

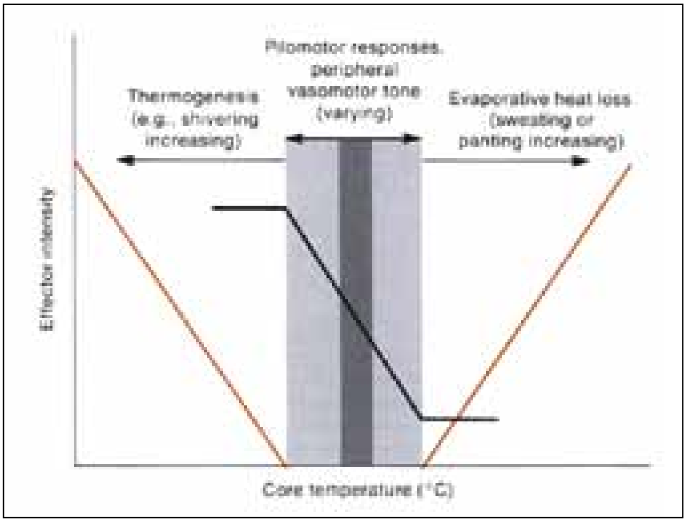


Figure 3 – The concept of concentric thermal shells – the brain, heart and lungs and other organs will constitute the core and will be maintained at or close to the set point deep body temperature (DBT) – the outer shells will be cooler and at the skin (outer shell) heat will be exchanged with the environment. Thermal imaging detects the surface temperature of the outermost shell.

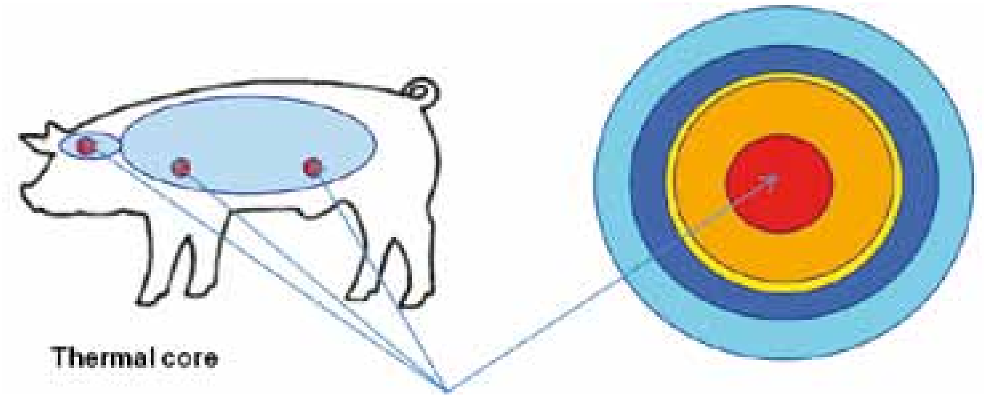


Figure 4 – Concentric thermal shells: The gradient from core to periphery increases during transition from hot to cold conditions – heat transfer form core to periphery decreases – insulation increases.

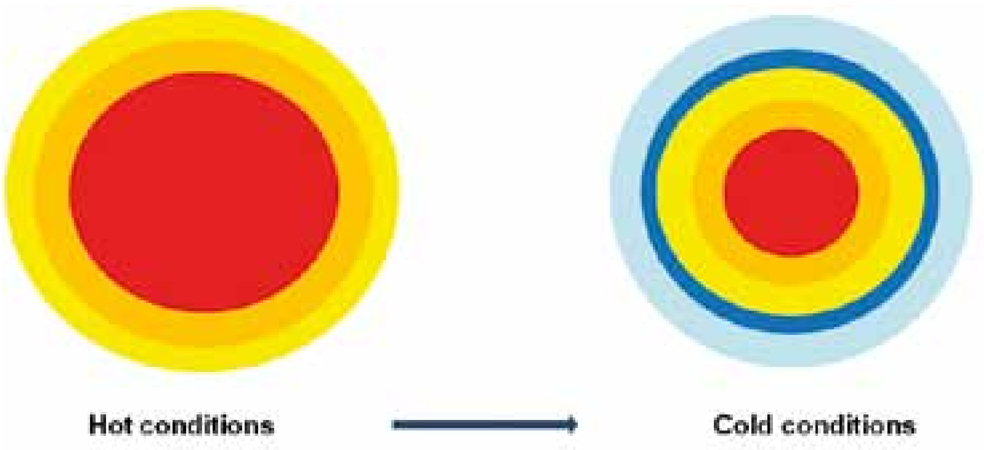


Figure 5 – Thermogram of a fully fleeced sheep under cold conditions in Scotland in winter. Note the contrast in surface temperature between the fleece and the less insulated facial features.

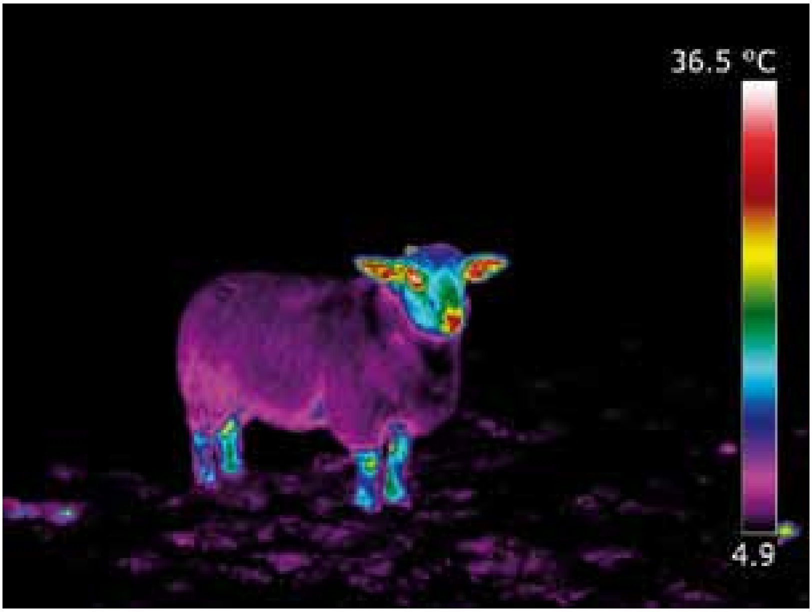


Figure 6 – The eye and inner canthus temperatures measured in a cow (lateral view) under thermoneutral conditions in a farm setting using a high resolution thermal imaging camera.

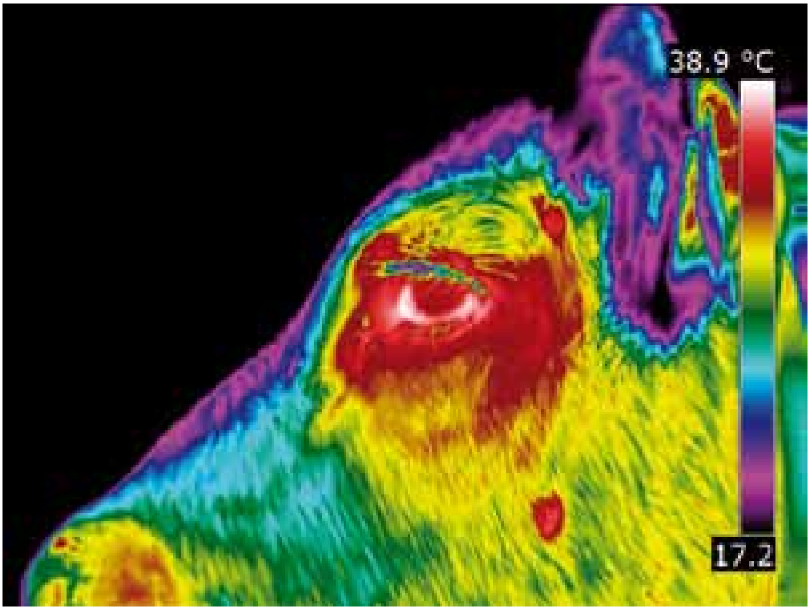


Figure 7 – Thermogram of the face and head of a cow to allow determination of regional surface temperatures in body temperature studies.

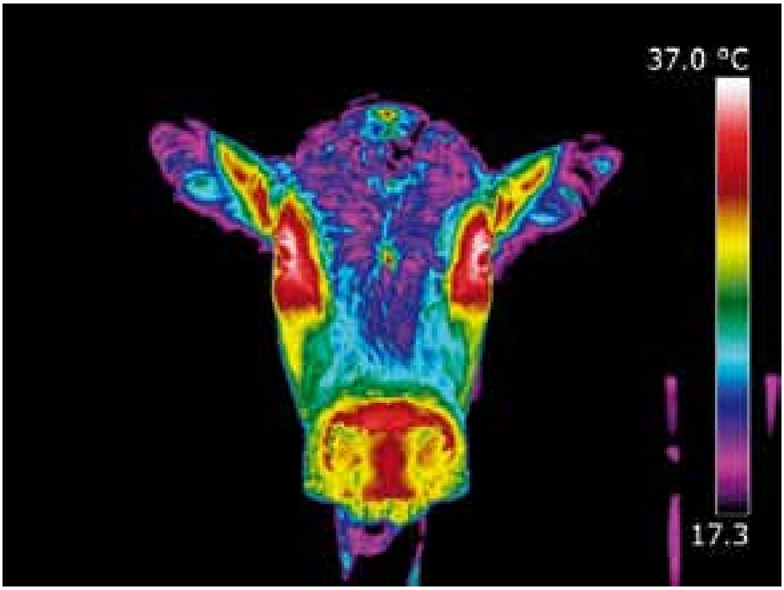


Figure 8 – Thermogram to determine the facial and head and neck temperature distributions in a young turkey in a commercial shed under cool winter conditions. The selection of a site for use in prediction of deep body temperature may be based upon identification of the maximum value (in areas of least feather insulation.

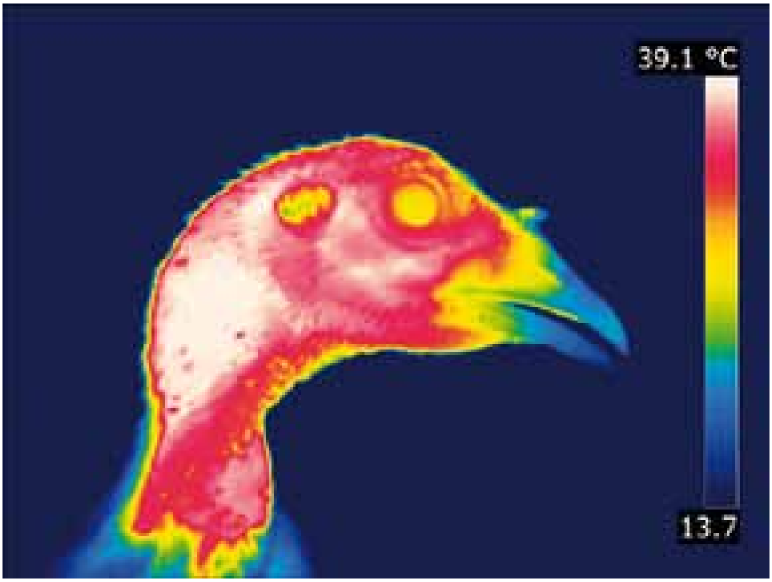


Figure 9 – Thermogram of the left side/flank of a cow – Note the cool areas where wetting has occurred as a result of the cow licking the coat.

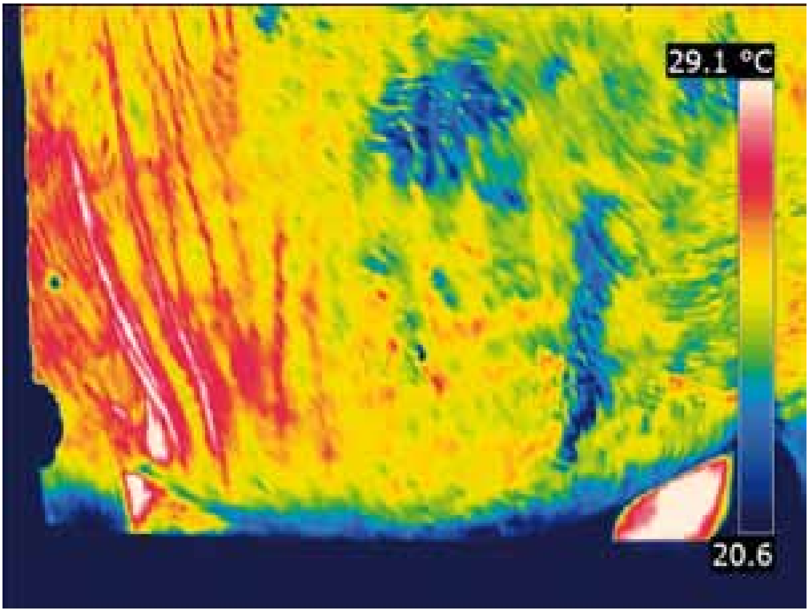
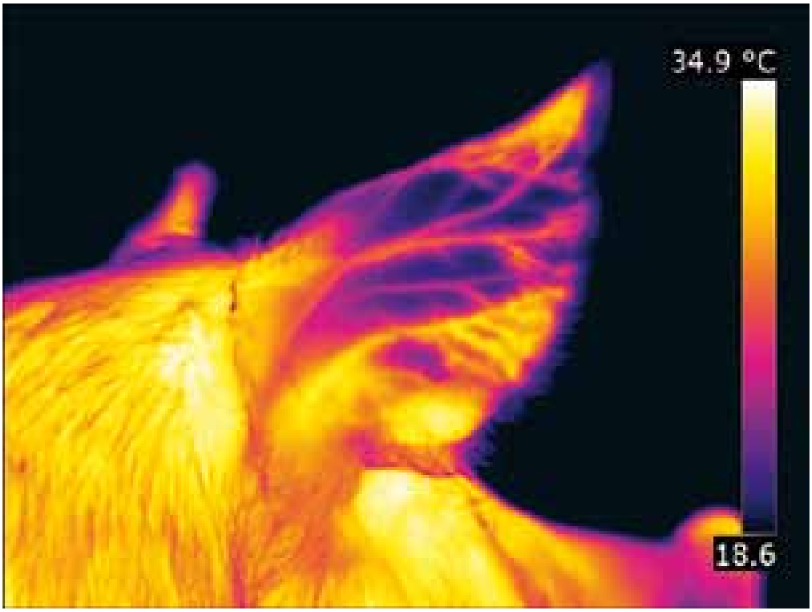
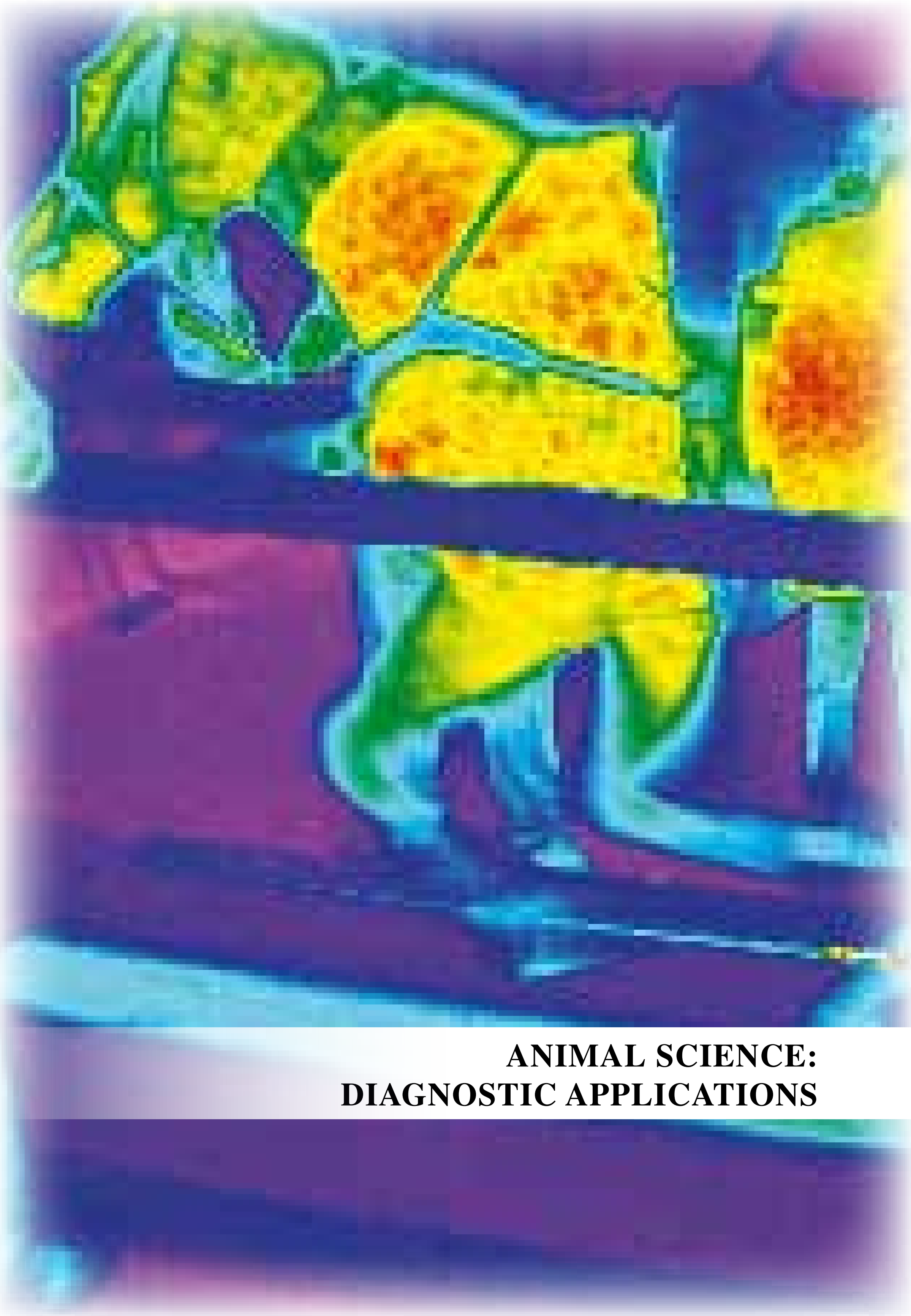


Figure 10 – Thermogram of a pig's ear to assess blood flow following administration of different sedative drugs during pre-surgery general anaesthesia.





ANIMAL SCIENCE: DIAGNOSTIC APPLICATIONS

HEAT GENERATION AND THE ROLE OF INFRARED THERMOGRAPHY IN PATHOLOGICAL CONDITIONS

ALLAN L. SCHAEFER[1], NIGEL J. COOK[2]

[1] Agriculture and Agri-Food Canada, Lacombe Research Centre, Alberta, Canada
[2] Alberta Agriculture, Lacombe Research Centre, Alberta, Canada

ABSTRACT

The use of thermal biometric measurements have been fundamental to identify disease states in both animals and humans throughout history. The thermogenesis of fever in pathological conditions is an interesting process and is known to be accompanied by an obligatory heat signature in the infrared spectrum. As a non-invasive, highly sensitive technology infrared thermography lends itself to the rapid, automated detection of early disease in animals. These characteristics have a significant advantage in modern agriculture where large numbers of animals in intensively raised groups are common.

Key words

Stress Physiology, Infrared Thermography, Early Disease Detection, Animal Welfare, Growth Physiology.

INTRODUCTION

The Council of Biology Editors (CBE Manual, 1994) describe the importance of obtaining precise scientific information to define how the natural world works. Such information and understanding has enabled our technological innovation and survival throughout history. In keeping with this understanding, in veterinary medicine, the treatment of a medical condition in an animal is almost always preceded by the provision of such scientific information via a diagnostic procedure. Indeed, pathology, the science dealing with the study and diagnosis of diseases is dependent on the provision of precise scientific information.

As described by Houdas and Ring in their 1982 review and more recently by Ring (2006; 2007) and Lahiri et al. (2012) it has long been recognized since the time of the ancient Greeks (400 BC) that one scientific item of information in particular, body temperature and the pathogenesis of fever, underpins many pathological conditions. The challenge in using thermal information within animal agriculture is twofold. Firstly, the patients are not always compliant and the act of capturing an animal in order to monitor its temperature can, in itself, bias the measurement due to the distress of restraint. Secondly, the scope and scale of modern agriculture with significant animal numbers presents challenges regarding how to efficiently collect accurate thermal information on larger numbers of animals. The purpose of the current chapter is to review some of the emerging opportunities to measure temperature information, specifically infrared thermography, to assist with the diagnosis of pathological conditions in animals.

HISTORICAL PERSPECTIVE

Well written historical reviews on the use of infrared thermography in animals do exist (Smith, 1964; Clark and Cena, 1972; Purohit, 2007) but their numbers have been limited. The number of research studies and reviews on the use of infrared thermography in both human medicine and animal agriculture has increased dramatically even in the past ten years (Stewart et al., 2005; McCafferty, 2007; Purohit, 2007). As reported by Neuberth (1993), Jones (1998), Jiang et al. (2005), Matsui et al. (2009) and Szentkuti et al. (2011) much of the increased interest in the use of infrared thermography has been due to improvements in cameras, reduced costs, and better computer aided diagnostics with user friendly platforms now produced by commercial companies (including FLIR, Indigo and Raytheon). Data assessment using newer multivariate analysis techniques such as those reported by Wang and Mizaikoff (2008), machine vision approaches (Guillaume-Alexandre-Bilodeau et al., 2011) and optical imaging (Signor et al., 2010) have been part of this development. In human medicine, these technological improvements have enabled the development of thermal texture maps (Yang and Yang, 2006) and medical reference libraries for neurovascular and musculoskeletal diseases and injury (Tkacova et al., 2011) and the study of SARS (Matsui et al., 2009). While such thermal reference libraries have been lacking, to date, for use in animal agriculture recent attempts to computerise thermal information in animals are emerging (Hovinen et al., 2008; Wirthgen et al., 2011; Schaefer et al., 2012).

PATHOGENESIS OF FEVER

Before discussing the concept of fever it is perhaps useful to first review the basic process of thermogenesis in an animal. The origin of the food derived energy in an animal is obtained through oxidative/reduction reactions during intermediary metabolic events as described in classical teaching texts on the subject such as that by Lehninger (1975). Much of the energy expenditure and hence heat production in an animal *per se* is thought to be the result of either protein synthesis or sodium pump activities (Lehninger, 1975; Schaefer et al., 1985; Nkrumah et al., 2006).

In their classic text on the subject of body temperature, Houdas and Ring (1982) described, over thirty years ago, how according to the first law of thermodynamics food energy in an animal can ultimately be converted to other alternative energy forms such as infrared radiation. This is consistent with the earlier description by Kleiber (1975) that much of the energy lost by an animal (40-60%) is lost through radiation in the infrared spectrum.

In homeothermic animals the temperature profile can be complex. Temperature distribution within an animal is not uniform (Houdas and Ring, 1982) and the core temperature of an animal is always changing. There are anatomical areas displaying higher heat characteristics, such as kidney, brain, myocardium, brown adipose tissue and liver due to higher rates of metabolic activity. Local tissue thermal gradients are even reported (Bleich and Moore, 1981) and can in themselves be diagnostic of pathological conditions. In addition, unique circadian rhythms in these temperature profiles can be seen (Houdas and Ring, 1982).

Normal temperature regulation in an animal can be equally complex with a host of physiological mechanisms engaged to control heat production, heat loss and heat retention including heat conduction across tissues, heat convection from a body surface, vascular counter-current mechanisms, vasoconstriction, vasodilation, piloerection, arterio-venous anastomoses, evaporative and respiratory cooling, hunting reactions (vascular dilation-constriction cycling), shivering and non-shivering thermogenesis (Robertshaw, 2004 ; Hales, 1984; Mount, 1979). As reported by these authors, in a homeothermic animal the monitoring, coordination and management of these mechanisms is via thermoreceptors located in the skin, body core, spinal cord and the central nervous system (CNS). However, it is the thermoreceptors located in the anterior hypothalamus that determine the basic "thermal set point" of the body temperature.

Physiological events including the activation of the hypothalamic-pituitary-adrenal axis and brown adipose tissue (Houdas and Ring, 1982) or even immune dysfunction (Algahtani and Mukundan, 2011) can increase body temperature. However, it is the presence and action of exogenous or endogenous pyrogens that initiate a fever condition. Fever is considered to occur when the core body temperature is elevated several degrees above the normal 37°C (human). Fever is basically brought about by the same thermoregulatory mechanisms notably vasoconstriction. Endogenous pyrogens originate in leukocytes and exogenous pyrogens are produced, for example, by gram negative bacteria. Exogenous pyrogens are reported to be lipopolysaccharides of high molecular weight. These pyrogens act on the preoptic area of the anterior hypothalamus and are known to be mediated by prostaglandins (Houdas and Ring, 1982).

A description of the fever induction process has been well described by several authors (Szelenyi, 1983; Dinarello, 1996; Mihail et al., 1998; Netea et al.,1999; Babcock Cimpello et al, 2000; VanReeth, 2000). Fever, or the increase in core body temperature above the normal 37°C, is described as part of an acute phase reaction. A number of events including infection, trauma, inflammation and some malignant diseases can induce an acute phase response depicted by, or associated with, a fever. These cytokine initiated pathological states are not to be confused with non-cytokine factors that can also increase core temperature such as exercise or heat stroke.

Infections are the most common cause of a fever and initiate a pyrogen induced cytokine response. As reported by Babcock Cimpello et al. (2000) and Netea et al (1999) the classical model of the pathogenesis of fever due to exogenous pyrogens is that the process is initiated by a cytokine pyrogen, typically interleukin 6 (Il-6), originating from leucocytes in the blood. The pyrogen causes the synthesis of prostaglandins as the mediator of a fever response acting again on the hypothalamus. However, there is also evidence that local production of cytokines can occur directly at the hypothalamus and hence multiple induction mechanisms for a febrile response may exist. A fever will also cause a cascade of acute phase protein changes within 12h of infection (Thurnham and McCabe 2012). These can include the production of ceruloplasmin, haptoglobin, C-reactive protein, amyloid A and fibrinogen.

Fever exists because an increase in body temperature can moderate the pathogenesis of an infection. As described by Bleich and Moore (1981) all known infectious agents are sensitive to temperature and host disease defences are temperature sensitive. This was observed in humans during the early diagnosis of syphilis and arthritis (Bleich and Moore, 1981). Part of the reason for this apparent benefit of a fever is likely due, in part, to the fact that a fever is reported to enhance hematopoiesis, leukocytosis, phagocytosis and antibody production. This position is further supported by the work of Babcock Cimpello (2000) reporting that fever can enhance neutrophile function and antimicrobial action by improving chemotactic responses and by increasing the production of superoxide and interferon. The fever process augments T-cell proliferation and enhances both cellular and humoral immunity. It has also been observed that suppressing fever can be associated with increased mortality (Babcock Cimpello et al 2000). However, these apparent advantages of a fever must be kept in balance. Unchecked, fever and hyperthermia can lead to permanent tissue damage, increased morbidity and even death. Therefore, there is a benefit to managing fever. Knowing the presence of, and identifying a fever response early during the onset of a pathological condition can enable a management process to be initiated earlier. Therein lies the value of utilizing infrared thermography as an early disease detection technology.

SENSITIVITY OF INFRARED THERMOGRAPHY

The accuracy and precision of thermal biometric measurements are important. In this respect, current technology for measuring both conductive measures of core body temperature and radiated temperatures are quite good. However, the sensitivity is another matter. In a homeothermic animal all the thermoregulatory mechanisms are designed to maintain a constant and stable core body temperature. In order to accomplish this, the animal will utilize and manage other thermal loss mechanisms including radiated energy in the infrared range to maintain a stable core temperature. Hence, when an animal is challenged with a disease condition the core temperature will often be the last thermal biometric to change and will be preceded by changes in other thermal heat loss mechanisms. This can be seen in the following example where weaned beef calves were monitored for three days post arrival at a feedlot. Seven of these calves were confirmed to be afflicted by bovine respiratory disease on day three. Thermal data for radiated temperature were obtained with the use of an Inframatics 740 broadband camera (Boston, USA) and core temperature was obtained using a conventional rectal thermometer. As seen in the following table, the proportional change in temperature or the sensitivity was substantially greater with the infrared measurement.

Table 1: Infrared and rectal temperature in weaned and transported calves (mean live weight 250 kg) received at a feedlot in Alberta. These seven calves were verified by clinical scores to be experiencing bovine respiratory diaease on day 3 post arrival.

N=7 calves	Infrared Temperature °C	Rectal Temperature °C
Day 1	21.88	39.4
Day 3	29.03	39.9
Change in degrees °C	7.14	0.5
% Change	33%	0.8%

IMAGE REFERENCES

Utility of Technology

As reported by Jiang et al (2005) and Lahiri et al. (2012) the primary advantages for using infrared thermography in humans to aid in the diagnosis of pathological conditions include the fact that the technology is non-invasive, is painless to apply or use, can be remotely operated, diagnostics can be made rapidly and in real time, the technique is safe and the cost of the equipment is competitive with other diagnostic approaches. These advantages apply equally well to use with animals. In addition, as mentioned earlier, with animals there is also the issue of having to capture and restrain an animal in order to use a conventional diagnostic procedure or to collect a biological sample for analysis. With infrared thermography, the fact that the technology can be used non-invasively has a significant advantage. In addition to these advantages, it would be noteworthy also that for many animals including cattle and swine, there are in place, or proposed regulatory requirements in North America, Europe and Australasia to identify each individual with a radio frequency identification tag operating on FM radio frequency. As described by Schaefer et al. (2012) this feature lends itself well to being used for animal identification in conjunction with an infrared camera. This approach thus makes the real time assessment of large numbers of animals feasible. Furthermore, this system can be computerised and made internet compatible as a disease surveillance management tool for producers and veterinarians (Schaefer et al 2012).

SPECIFIC APPLICATIONS IN VETERINARY MEDICINE

The following figures are examples of how infrared thermography can be used to identify disease conditions in animals.

Additional examples of the use of infrared to study pathological conditions in bovine respiratory disease in beef cattle (Schaefer et al. 2004, 2005, 2007), mastitis in dairy cattle (Colak et al., 2008; Hovinen et al., 2008), pale soft exudative pork in swine and tracheal laryngitis in poultry (see Cook and Schaefer Chapter).

REFERENCES

1) Algahtani, M. and Mukundan, D. "Current understanding of fever and host immunity". Curr. Opin. Pediatric. 23: 115-120. 2011.
2) Alsaaod, M. and Buscher, W. " Detection of hoof lesions using digital infrared thermography in dairy cows". J. Dairy Sci. 95(2): 735-742. 2012.
3) Babcock Cimpello, L., D.L. Goldman and H. Klhine. " Fever pathophysiology". Clin. Ped. 1: 84-93. 2000.
4) Bleich, H.L. and Moore, M.J. " The role of regional body temperature in the pathogenesis of disease" . New England J. Med. 305(14): 808-814. 1981.
5) CBE Manual. Scientific Style and Format. 6th Ed. Cambridge University Press. New York. 1994.
6) Clark, J.A. and Cena, K. " Thermographic measurements of the surface temperatures of animals. J. of Mammology. 54: 1003-1007. 1972.
7) Colak, A., B. Polat, Z. Okumus, M. Kaya, L.E. Yanmaz and A. Hayirli. " Early detection of mastitis using infrared thermography in dairy cows". J. Dairy Sci. 91(11): 4244-4248. 2008.
8) Dinarello, C.A. "Thermoregulation and the pathogenesis of fever". Infection and Disease Clinics of North America. 10(2): 433-449. 1996.
9) Guillaume-Alexandre Bilodeau, A. Torabi, M. Levesque, C. Ouellet, J.M.P. Langlois, P. Lema and L. Carmant. " Body temperature estimates of a moving subject from thermographic images " . Machine Vision and Applications DOI. 10: 1007. 2011.
10) Hales, J.R.S. " Thermal Physiology". Raven Press. New York. 1984.
11) Houdas, Y. and Ring,E.F.G . " Human body temperature". Plenum Press. N.Y. 1982.
12) Hovinen, M., J. Sivonen, L. Taponen, L. Hanninen, M. Pastell, A.M. Aisla and S. Ptorela. "Detection of clinical mastitis with the help of a thermal camera". J. Dairy Sci. 91(12): 4592-4598. 2008.
13) Jones, B. "A reappraisal of the use of infrared thermal image analysis in medicine". IEEE Transactions on Medical Imaging. 17(6): 1019-1027. 1998.
14) Jiang, L.J., E.Y.K. Ng, A.C.B. Yeo, S. Wu, F. Pan, W.Y. Yau, J.H. Chen and Y. Yang. 'A perspective on medical infrared imaging ' . J. of Med. Eng. And Tech. 29(6): 257-267. 2005.
15) Kleiber, M. " The fire of life". R.E. Krieger Pub Comp. 1975.
16) Lahiri, B.B., S. Bagavathiappan, T. Jayakumar and J. Philip. ' Medical applications of infrared thermography. A Review" . Infrared Physics and Technology. 55: 221-235. 2012.
17) Lehninger, A.L. " Biochemistry. Catabolism and the generation of phosphate bond energy" 363-616 and " Active transport across membranes" 779-805. Worth Publishers Inc. N.Y. 1975.
18) Matsui, T., Suzuki, S., Ujikawa, K., Usui, T., Gotoh, S., Sugamata, M., Badarch, Z. and Abe, S. " Development of a non-contact screening system for rapid medical inspection at a quarantine depot using a laser Doppler blood flow meter, microwave radar and infrared trhermography". J of Medical Engineering and Technology. 33(5): 403-409. 2009.

19) McCafferty, D.J. “The value of infrared thermography for research in mammals. Previuos applications and future directions”. Mammal. Rev. 37(3): 207-223. 2007.
20) Mihail, G. N., B.J. Kullberg and J.W.M. van der Meer. “Pathogenesis of fever” . Clinical Infectious Diseases. 26: 1479. 1998.
21) Mount, L.E. “ Adaptation to thermal environment. Man and his productive animals. Edward Arnold Pub Ltd. London. UK. 1979.
22) Netea, M., B.J. Kullberd and J.W.M. Van der Meer. “Do only circulating pyrogenic cytokines act as mediators in the febrile response. A Hypothesis”. Eur. J. Clin. Invest. 29(4): 351-356. 1999.
23) Neuberth, M. “Imaging methods in veterinary medicine”. Bildgebende Verfahren in der Veterinamedizin. 68-72. 1993.
24) Nkrumah, J.D., E.K. Okine, G.W. Mathison, K. Schmid, C. Li, J.A. Basarab, M.A. Price, Z. Wang and S.S. Moore. Relationships of feedlot feed efficiency, performance, and feeding behaviour with metabolic rate, methane production and energy particioning in beef cattle. J. Anim. Sci. 84. 145-153. 2006.
25) Purohit., R.C. “ History and research reviews of thermology in veterinary medicine at Auburn University”. Thermology Int. 17(4): 127-132. 2007.
26) Robertshaw, D. “Temperature regulation and the thermal environment”. P 962-973. In Dukes Physiology of Domestic Animals. Ed By W.O. Reece. Comstock Pub Associates. N.Y. 2004.
27) Ring, E.F.J. “The historical development of thermography and thermal imaging in medicine”. J. Medical Engineering and Technology. 30(4): 192-198. 2006.
28) Ring, E.F.J. “The historical development of temperature measurement in medicine. Infrared Physics and Technology. 49: 297-301. 2007.
29) Schaefer, A.L., Davis, S.R. and Hughson, G.A. “The importance of protein turnover to metabolism and carcass composition of growing lambs”. Proc. New Zealand Soc. Anim. Prod. 45: 85-88. 1985.
30) Schaefer, A.L., Cook, N., Tessaro, S.V., Deregt, D., Desroches, G., Dubeski, P. L., Tong, A.K.W. and Godson, D.L. “Early detection and prediction of infection using infrared thermography”. Can. J. ANim. Sci. 84: 73-80. 2004.
31) Schaefer, A.L., Perry, B.J., Cook, N.J., Miller, C.C., Church, J.S., Tong, A.K.W. and Stenzler, A. “ Infrared detection and nitric oxide treatment of bovine respiratory disease”. Online J. Vet. Res. 10(1): 7-16. 2005.
32) Schaefer, A.L., Cook, N.J., Church, J.S., Basarab, J., Perry, B., Millar, C. and Tong, A.K.W. “The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves”. Res. Vet. Sci. 83: 376-384. 2007.
33) Schaefer, A.L., Cook, N.J., Bench, C., Chabot, J.B., Colyn, J., Liu, T., Okine, E.K., Stewart, M., and Webster, J.R. “ The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography”. Res. Vet. Sci. 93: 928-935. 2012.
34) Smith, W.M. “ Applications of thermography in veterinary medicine”. Annal. Of the New York Academy of Sci. 121: 248-254. 1964.
35) Signor, A., Mather, S.J., Piaggio, G., Malviya, G. and Dierckx, R.A. “ Molecular imaging of inflammation/infection: Nuclear Medicine and Optical Imaging Agents and Methods”. Chem Rev. 110: 3112-3145. 2010.
36) Spire, M. , Drouillard, J. S., Galland, J. C. and Sargeant, J. M. “ Use of infrared thermography to detect inflammation caused by contaminated growth promotant ear implants in cattle”. J. Amer. Vet. Med. Assoc. 215(9): 1320-1324. 1999.
37) Stewart, M., Webster, J.R., Schaefer, A.L., Cook, N.J. and Scott, S.L. “ Infrared thernmography as a non invasive tool to study animal welfare. Anim. Welf. 14: 319-325. 2005.

38) Szelenyi, Z. " Current views on the pathogenesis of fever". J. Thermal. Biol. 8: 185-190. 1983.
39) Szentkuti, A., Kavanagh, H. S. and Grazio, S.. " Infrared thermography and image analysis for biomedical use:. Periodicium Biologorum. 113(4): 385-392. 2011.
40) Thurnham DI, McCabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010*. Geneva, World Health Organization, 2012.
41) Tkacova, M., Hudák, R., Živčák, J. and Sidun, J.. " Thermographic atlas of the human body". IEEE. 2011.
42) VanReeth, K. "Cytokines in the pathogenesis of influenza". Vet. Microbiol. 74: 109-116. 2000.
43) Wang, L. and Mizaikoff, B. "Applications of multivariate data analysis techniques to biomedical diagnosis based on mid-infrared spectroscopy". Anal. Bioanal. Chem. 391: 1641-1654. 2008.
44) Wirthgen, T., Zipser, S., Franze, U., Geidel, S. and Zimmerhackl, M. "Automated infrared based health monitoring system for veterinary application". Procedia Engineering. 25: 1405-1408. 2011.
45) Yang, W.J. and Yang, P.P.T. "New technology of functional infrared imaging and its clinical applications". 2006.

Figure 1: Pododermatitis (Foot Rot). Infrared image of an infected left hind claw of a beef bull. Suspect Fusobacterium necrophorum or Bacteroides Melaninogenicus. Laminitis also described by Alsaaod and Buscher 2012.

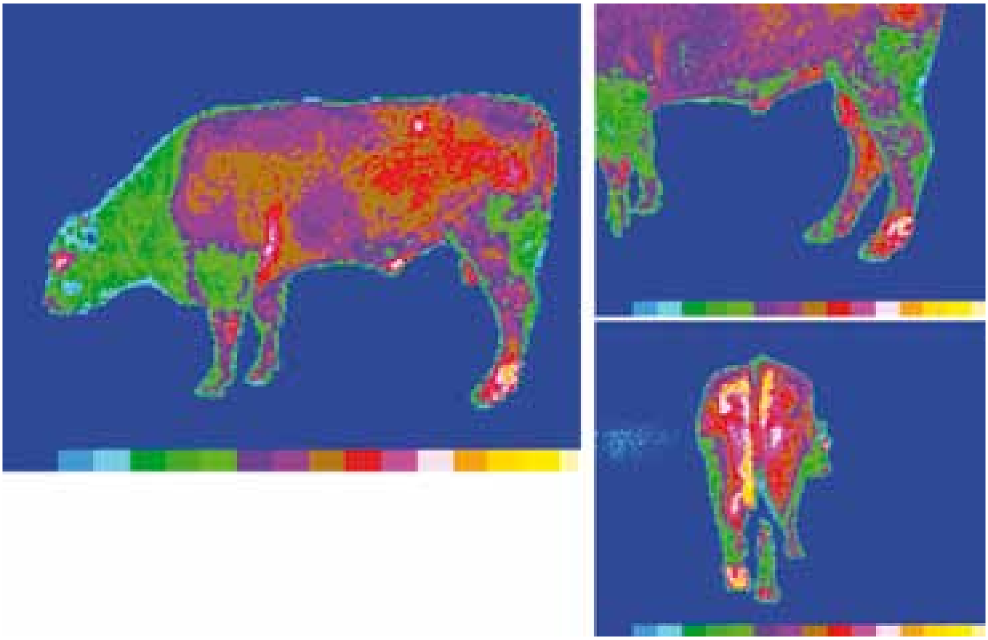


Figure 2: Illustration of an infected ear in a pig.

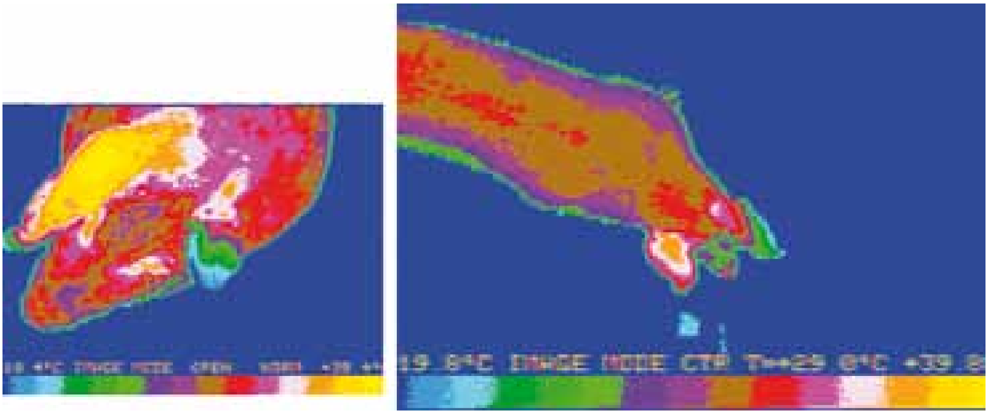


Figure 3: Illustration of infrared thermography used to non invasively study heat loss in a population of wild animals (Muskox on Banks Island, NWT, Canada. Image taken by P. Lepage, Agriculture and Agri-Food Canada, Lacombe Research Centre, Alberta, Canada).

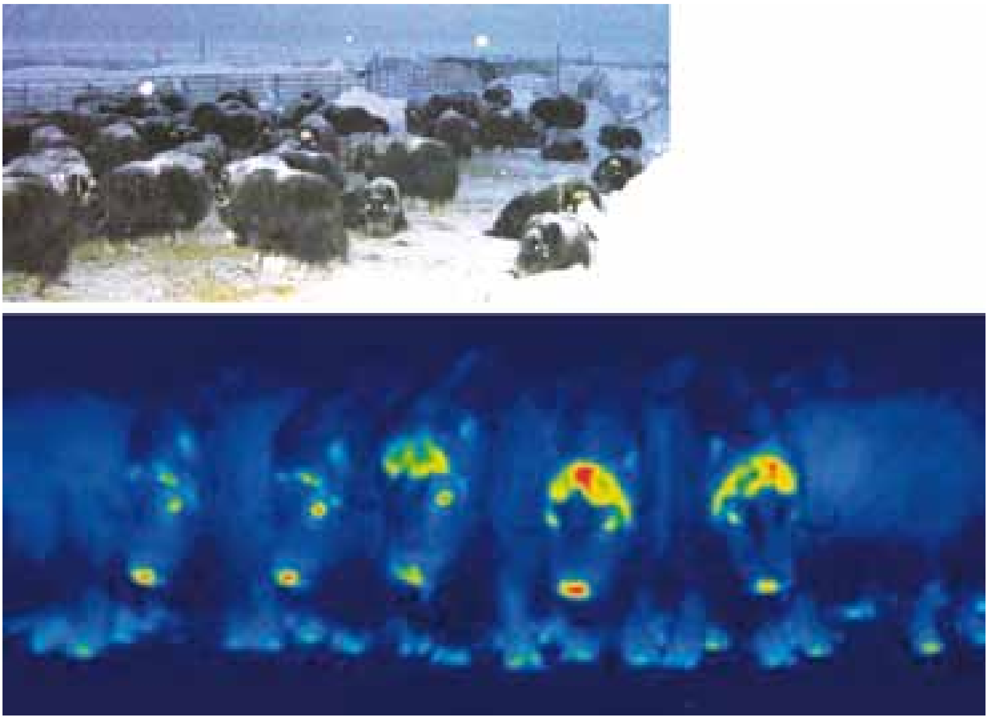


Figure 4: Illustration of infrared thermography used to study transport stress in domestic Emu

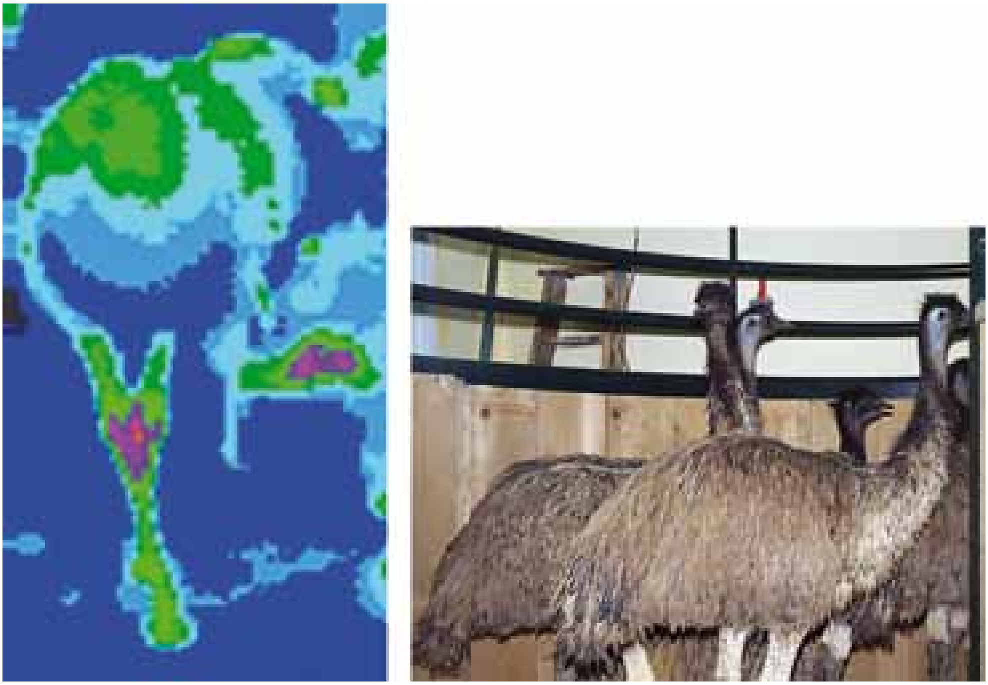


Figure 5: Illustration of infrared thermography used to study transport stress in Bison

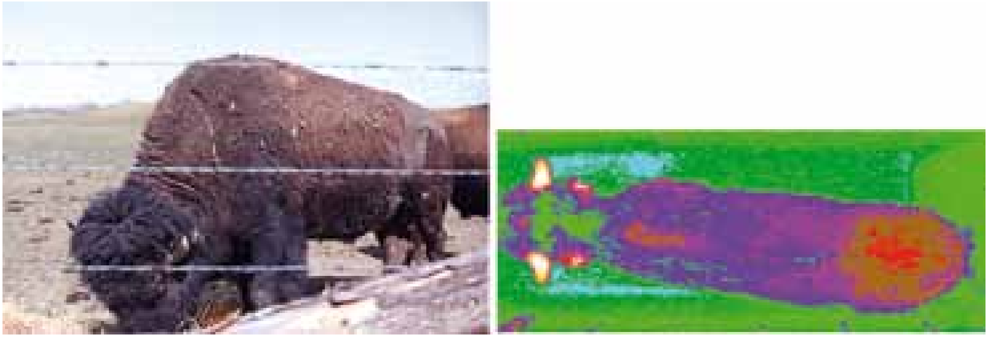


Figure 6: Illustration of infrared thermography used to study transport stress in Elk

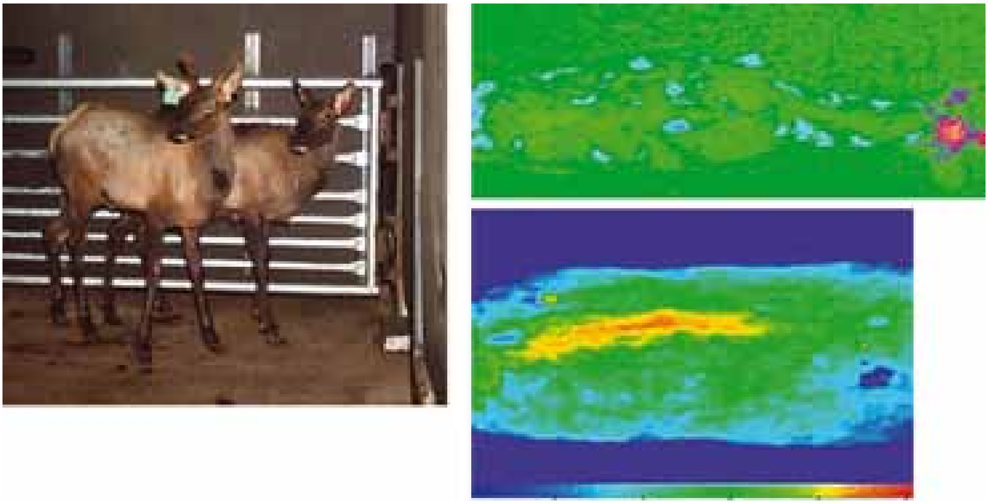
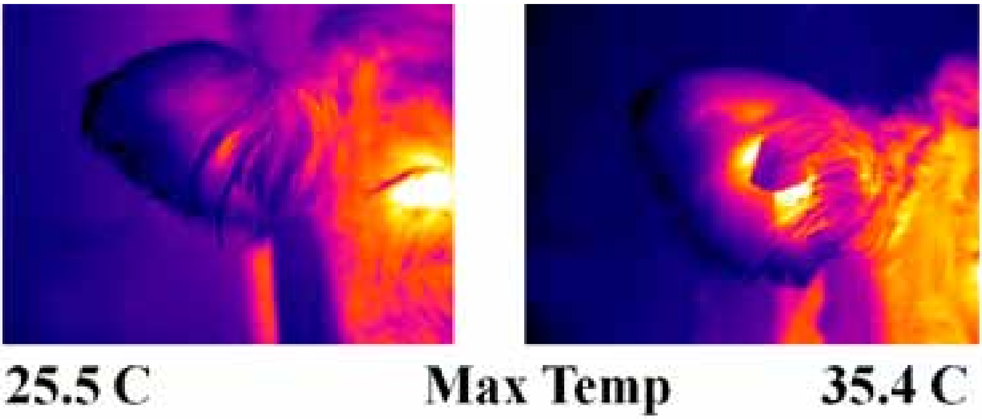


Figure 7: Infected ear tag in a beef calf. (Technique also reported by Spire et al., 1999).



INFRARED THERMOGRAPHY AND DISEASE SURVEILLANCE

NIGEL J. COOK[1], ALLAN L. SCHAEFER[2]

[1] Alberta Agriculture, Lacombe Research Centre, Alberta, Canada
[2] Agriculture and Agri-Food Canada, Lacombe Research Centre, Alberta, Canada

ABSTRACT

Infrared thermography (IRT) can be used to detect febrile disease in livestock species. Disease surveillance can entail single-time point measurements of transient populations, or multiple time-point measurements of static populations. Animals in the food chain are moved from farm to abattoir, often passing through intermediate facilities such as auctions or feedlots that provide an opportunity for single-point screening for febrile disease. Alternatively, intensely housed livestock represent static populations that can be continually monitored by infrared thermography. Dairy cattle and swine provide excellent examples of this type of surveillance. Diagnostic test performance varies greatly between these types of disease surveillance applications of IRT. A third type of surveillance uses IRT to identify the exposure of livestock animals to disease vectors such as rodents, thereby providing a method of estimating the risk of disease transmission.

Key words

Infrared thermography, disease surveillance, febrile disease, diagnostic test performance.

Infrared thermography (IRT) is a non-contact, and therefore remote, method of measuring the surface temperature of humans and animals. This fact gives rise to the concept of IRT as a method of disease surveillance. A considerable amount has been written concerning the potential of IRT for disease surveillance but in reality there are very limited numbers of practical examples of the application of the technology in this context. It is important to define what precisely is meant by disease surveillance because the technology is applied in different ways depending on the situation. Most often, disease surveillance refers to an epidemiological assessment whereby outbreaks of disease are tracked. Perhaps the best known example of IRT in this context is the use of thermal cameras to scan airline passengers during the pandemic outbreaks of SARS in 2003 and the H1N1 ‘swine flu’ of 2009. Temperature measurement by IRT is made once for each individual in a large group, and a decision on identification of febrile disease is made in real time. Although the practical examples of this type of surveillance are of human subjects the technology applies to livestock species in much the same ways.

In a broader context, surveillance by IRT can be used on a static population where a group of subjects is continuously or repeatedly monitored. In these situations temperature measurement can be made on the group as a whole, or on individuals within the group. This type of surveillance is most appropriate for livestock animals in confined settings such as poultry, swine and dairy barns, or feedlot settings for beef cattle. Perhaps the best examples of disease surveillance in this context are the automated monitoring of dairy cattle for mastitis, and feedlot cattle for respiratory disorders.

In both these contexts IRT is used to measure surface temperature and infers pathological conditions from detection of aberrant temperature or to variation in temperature measurements. However, the number of temperature measurements, whether single or multiple, places different emphases and expectations on the diagnostic utility and efficacy of IRT. Use of the technology for mass scanning of transient populations utilizes a single, fixed temperature as a referent value for deciding a positive test. The expectation in this context is that measurement by IRT will reflect core temperature and thereby detect individuals exhibiting febrile disease. Multiple temperature measurements permit a more accurate assessment of thermal regulation in response to physiological challenges. Thus, changes in radiated temperature may reflect an organism's attempts to maintain core temperature in the face of those challenges. As a consequence, radiated temperature may not necessarily reflect core temperature but nonetheless be a superior indicator of thermal challenge because it reflects an animal's attempts at thermoregulation. This is important in the early diagnosis of disease prior to the onset of other symptoms such as fever.

A potential third surveillance application of IRT is the identification of species that come into contact with either humans or livestock and act as a reservoir for zoonotic diseases. In this respect, perhaps the greatest potential is in intensive housing environments. An important reservoir of zoonotic diseases of swine and poultry farms worldwide is rodents, particularly the house mouse (*Mus musculus*) and the brown rat (*Rattus norvegicus*) (Backhans and Fellstrom, 2012). The presence of mice in swine pens is relatively easy to identify using IRT (Figure 1), and could provide an estimate of the frequency of contact between rodents and livestock, and thereby a measure of risk for transmission of zoonotic diseases. This type of risk assessment was conducted by Witmer et al. (2010) who used IRT to identify the livestock species coming into contact with cattle during an outbreak of bovine tuberculosis.

In a review of the medical applications of IRT in humans, Lahiri et al. (2012) claim that mass screening for fever using IRT is an effective method of controlling the spread of pandemic disease such as SARS and influenza, and cite several references to support this supposition. However, closer examination of these studies reveal that mass screening using IRT may not be as efficacious as claimed.

Lutz et al. (2011) reported on the "mass screening of production animals for the early detection of febrile diseases" and concluded that IRT holds promise as a mass screening tool. However, the paper merely compared different methods of measuring the temperature of a group of cattle, and reported that IRT gave equivalent information to more conventional methods of temperature measurement. This is a long way from empirically demonstrating that IRT has efficacy as a mass screening tool for febrile disease, and much work remains to be done in this area. The major reasons why it is difficult to assess the efficacy of IRT for disease surveillance by comparisons among different studies include the following.

Surveillance applications

As stated above, one-time measurements on transient populations *vs.* multiple measurements on static populations have different expectations for test performance. Multiple measurements increase the diagnostic efficacy of temperature measurement by IRT.

Methods of obtaining IRT images

The distance and angle of the subject to the camera have profound effects on the accuracy and precision of temperature measurement by IRT. Unfortunately, methods of collecting thermal images vary considerably between applications. Measurements on human subjects in public places such as hospitals and airports are conducted as briefly as possible. Ng et al. (2004) and Nguyen et al. (2010) took measurements on hospital patients that were briefly posed in front of a hand-held camera, with fixed subject to camera distance and angles. Chiu et al. (2005) made measurements in a hospital setting on moving subjects as they approached a fixed camera, and Nishiura and Kamiya (2011) report on a similar method of application at the Narita International Airport, Japan during the H1N1 pandemic of 2009. In both these reports the distances and angles between the subjects and the camera were unspecified. Chiang et al. (2008) employed the same method in a hospital setting but specified subject to camera distances. Even within one application thermal images can be collected in more than one way. In the evaluation of screening procedures in Japan during the H1N1 pandemic Sakaguchi et al. (2012) report that during the early stages of the pandemic, airplane passengers were scanned on the aircraft by an operator with a hand-held camera, but in later stages the cameras were placed in fixed positions within the airport terminal. More accurate and precise measurements are recorded if the subjects are posed in front of a camera that is kept in a fixed position (Ring et al., 2008). In animal studies the subjects are often relatively stationary such as dairy cattle during milking (Kunc et al., 2007), or beef cattle in a pneumatic squeeze (Schaefer et al., 2004, 2007), or at water station (Schaefer et al., 2012). In all applications it is preferable if the conditions for recording thermal images can be kept as stable as possible.

Camera manufacturers and models

Nguyen et al. (2010) showed that temperature referent values providing optimum test efficiency for screening of hospital patients for febrile disease varied between 31.8°C to 35.8°C depending on the make of camera from different manufacturers**.** This study compared three cameras from different manufacturers and thus, given the numbers of different makes and models on the market, the variation between cameras can be expected to be higher than that reported by Nguyen et al. (2010). Even with a single camera, the measured temperature can be affected by drift in the automatic recalibration of the camera (Ng et al., 2004). The lack of standardization in thermal cameras is highly problematic for assessing of the efficacy of mass screening by IRT (Pascoe et al., 2010), and has led to the development of minimal standards for use with human subjects (IEC 80601-2-59:2008, Medical electrical equipment - Part 2-59 and International Organization for Standardization ISO/TR 13154:2009). These standards lay out technical and operational parameters for temperature measurements and specify such parameters as the camera sensitivity (0.1°C) and precision (±0.5°C) and the numbers of pixels in the image relating to the anatomical location of the target area for temperature measurements. No such standardization exists for applications in animal species but many of the proposed standards for use with humans are directly applicable to animal species.

Anatomical locations

The sensitivity of IRT temperature measurements to detect febrile disease depends on the anatomical location of the measurement. Many anatomical locations have been used, often depending on the species and occasionally according to a specific application such as measuring udder temperature for detection of mastitis in dairy cattle. Images of the head are most often captured for surveillance applications, and the corner of the eye (canthus) is becoming the most consistently used location for surveillance of humans (Ng, 2005; Ring et al., 2008; Pascoe and Fisher, 2010). Similarly, the canthus of the eye was shown to give the best results for prediction of bovine viral diarrhoea (BVD) in cattle compared to the nose, ear, hoof, dorsal and lateral views (Schaefer et al., 2004) (Figure 2). In ponies this area was shown to correlate significantly with rectal temperature. Using a rectal temperature of >38.6°C as a referent for febrile disease the IRT measurement of the canthus temperature gave a test sensitivity and specificity of 74.6% and 92.3%, respectively (Johnson et al., 2011). The sensitivity of this location is probably because blood flow is nearer to the surface at this point than at any other area of the face for humans and animals and as such is a more accurate reflection of core body temperature than other locations (Ng et al., 2004).

Environment conditions

The measurement of surface temperature is influenced by environmental conditions (Berry et al., 2003; Ng et al., 2004; Chiang et al., 2008; Nguyen et al., 2010). The environmental effect on temperature measurement can be accounted for in some cameras by automatic recalibration to ambient temperature, or by manual calibration (Nguyen et al., 2010). The most accurate and precise measurements are achieved when the environmental conditions can be stabilized within specified limits (Schaefer et al., 2004: Pascoe et al., 2010). Even under relatively stable conditions such as inside poultry, swine and dairy barns, there is probably sufficient environmental variation to affect temperature measurement. This problem is much greater for animals in an open environment due to effects such as solar loading, wind chill or wet surfaces. Algorithms that include measurement of environmental parameters improve the accuracy for measuring animal temperature. Berry et al. (2003) showed that the most accurate prediction of udder surface temperature relating to early detection of mastitis in dairy cattle was achieved by a combination of previous udder temperatures with environmental temperatures.

Choice of test referents and measures of test performance

Any assessment of the efficacy of IRT for disease surveillance includes measures of diagnostic test performance. Most assessments include test sensitivity and specificity, but should also include positive predictive value (PPV), negative predictive value (NPV) and test efficiency. It is important to note that the measures of test performance are dependent on the referent value of the test procedure, and that test sensitivity and specificity are inversely related. In mass screening applications it is often very important to detect all instances of an infectious disease in order to limit the spread. In this scenario, the test referent temperature is set relatively low in order to provide high test sensitivity. However, the trade-off for high sensitivity is a reduction in test specificity, and consequently an increase in the numbers of false positive test results. Since mass screening inevitably involves large numbers of individuals the numbers of false-positive tests may be very high. Since any positive test requires further actions such as confirmatory testing, or possible isolation (quarantine) and treatment, the costs for follow-up actions can be very substantial in terms manpower and resources. Diseases that pose a high risk of transmission and/or morbidity require surveillance tests with a high sensitivity. However, for those diseases that have low morbidity and low risk of transmission the surveillance test might favour specificity, thereby curtailing the follow-up costs. In practice, the choice of test referent often falls somewhere between these extremes with a test referent giving the highest efficiency, *i.e.* the proportion of test results that are true results, whether positive or negative.

Despite the seemingly widespread belief that IRT has application for disease surveillance, the use of IRT in this context is in its infancy. There are only a limited number of research papers that have attempted to systematically assess the efficacy of IRT for disease surveillance and all of these are concerned with surveillance of diseases important to human medicine. The difficulty in obtaining an accurate assessment of the efficacy of IRT for disease surveillance is highlighted in a review by Bitar (2009). In studies of screening of hospital patients by IRT, the test sensitivity ranged from 4% to 89.6%, specificity was 75.4% to 99.6%, and when prevalence was fixed at 1% the PPV ranged from 3.5% to 65.4%. The problem for assessing the efficacy of IRT in surveillance applications involving mass screening of large populations arises from the many ways in which the technology has been applied and the results interpreted. In addition, focusing on the performance of IRT for disease surveillance can give a false impression of its usefulness, because it is often evaluated in terms of a standalone test. In reality, optimal utilization of this technology is achieved if it is regarded as one tool among several, with the objective of focusing-in on infected individuals by a progressive reduction in the population to which the tests are serially applied. An example is the screening of airport passengers entering Japan during the SARS pandemic in which initial screening was conducted by questionnaire followed by a thermal measurement by IRT (Sakaguchi et al., 2012). A similar approach could be used in animals to improve the efficacy of IRT by combining temperature measurement with other methods of health assessment such as body condition scores and clinical symptoms, or known risk factors such as prior transport conditions and management practices such as back-grounding. The one aspect of screening of human subjects that is not applicable to mass screening of animals is that people can be deceitful in order to avoid detection. Despite the variations in the reported efficacy of the application nearly all authors report that IRT is a useful tool for limiting the spread of pandemic diseases.

DISEASE SURVEILLANCE BY MULTIPLE TEMPERATURE MEASUREMENTS

Most of the examples of disease surveillance, and as a consequence nearly all of the evaluation of diagnostic performance, have involved human subjects and single time-point measurements. There is opportunity for this type of application in the chain of production from farm to abattoir of livestock species. However, by far the most common application of IRT in animals involves multiple measurements on relatively stable populations over extended periods of time, *e.g.* dairy cattle. Consequently, the diagnostic performance can be improved greatly because multiple measurements provide a better assessment of the thermoregulatory response to physiological challenge and infection. In addition, multiple measurements allow a variety of test referents that might include either comparisons to previous measurements within individual animals, or comparison of an individual's thermal data to that of its cohorts in a group. More accurate and precise measures of thermoregulatory responses may also provide an indication of disease states prior to the onset of clinical symptoms. Multiple measurements are applied to static populations and can be made either on the group as a whole, or on individuals within the group by using radio frequency identification (RFID) tags.

MEASUREMENT OF INDIVIDUALS

Perhaps the best example of repeated temperature measurements by IRT of individual animals within a group is in the detection of mastitis in dairy cattle. Mastitis is the most important disease of dairy herds, incurring substantial costs to the dairy industry through loss of milk production and treatment costs. The severity of the disease ranges from sub-clinical to acute inflammation and is usually caused by bacterial infection of the teat canal. Sub-clinical infection is the most difficult to detect due to the absence of visible clinical symptoms but the costs to milk production are very high. It is important to diagnose mastitis as early as possible to increase the opportunity for a favourable treatment outcome and reduce the losses associated with a reduction in the quality and quantity of milk. Consequently, on-farm testing is a necessary requirement to reduce the economic impact of the disease. The search for a rapid, accurate and inexpensive test that can be conducted on-farm and is diagnostic of sub-clinical disease has been described as "the holy grail" for mastitis detection (Hillerton, 2003). These requirements have driven research efforts to include temperature measurements of the udder by IRT (Viguier et al, 2009).

A symptom of mastitis is heat generation from localized inflammatory reactions and thus IRT has the potential to be a rapid, non-invasive, real-time method of detecting mastitis. The structural layouts of many dairies, particularly the rotary types, lend themselves to the development of an automated system. Since dairy cattle are milked at least twice per day repeated measures on the same animal can be made and compared to prior measurements in that animal, or compared to measurements from the population of animals on the same farm. The earliest work in this field is that of Scott et al. (2000) who showed that infusion of *E. coli* lipopolysaccharide (LPS) into the udder of dairy cows increased the surface temperature of the infused quarter relative to the temperature of untreated quarters by approximately 2.3°C. Figure 3 shows the progression in temperature of the udder after infusion of LPS. Each image was analysed by tracing the outline of the udder and the temperatures given in Figure 3 are the maximum and average temperatures within the traced area. Note that both temperature parameters increased after induction but the largest relative increases occurred with the maximum image temperature. In a very similar experiment, Hovenin et al. (2008) infused *E. coli* LPS into the left forequarter of the udder, and showed that the treated quarter exhibited an increase in surface temperature of 1 - 1.5°C compared to the untreated right quarter. More recently, Pezeshki et al. (2011) conducted a similar experiment to compare several potential biomarkers of mastitis via induction with *E. coli* serotype O32:H37. Peak udder surface temperature was noted to increase 2 - 3ºC but lagged behind an increase in rectal temperature. Consequently, it was concluded that IRT measurement of udder surface temperature was not a useful marker of mastitis in dairy cattle. The increase in rectal temperature prior to udder temperature seems biologically implausible given that udder inflammation is a localised response and therefore would be more likely to occur prior to systemic effects. The discrepancy with previous findings may have been due to how the areas for temperature measurements were defined, and what measurement parameters were used to define a response, *e.g.* maximum *vs.* average temperature. In the study of Scott et al. (2000) the whole udder was traced and the temperature variables were the maximum and average temperature of this area. Hovenin et al. (2008) also used maximum image temperature derived from the whole udder surface in the image, but the mean temperature was determined from a circular area adjacent to the teats. By comparison, Pezeshki et al. (2011) used the mean temperature as the response parameter and this was obtained from a boxed area placed centrally on the udder surface. This latter approach may be less accurate because the positioning of the boxes may not correspond to areas of the udder that exhibit a significant thermal response. Furthermore, the maximum temperature variable provides the best parameter of response (Scott et al, 2000) and this might be easily missed if the maximum temperature was located on part of the image that was not inside the box. This effect was noted by Hovenin et al. (2008) when the maximum image temperature was not always within the circular area they defined for measurement purposes. These differences between research reports demonstrate how the efficacy of IRT can be determined by how the measurements are made. Berry et al. (2003) demonstrated a circadian rhythm in udder surface temperature as well as a relationship of udder temperature to environmental temperature. Combining these factors they were able to predict current udder temperature from previous temperatures and environment temperature. This observation suggested that the diagnostic referent may be an absolute temperature or a change in temperature from previous measurements, and that including environmental temperature was an important parameter.

In cases of mastitis, udder surface temperature as measured by IRT was shown to strongly correlate (r = 0.92) with the California Mastitis Test (CMT) (Colak et al., 2008). However, the diagnostic performance of IRT using CMT as the diagnostic standard was not reported, and temperature measurements were not corrected for variables such as time of day (circadian rhythmicity) or environmental temperature. Thus, although the study was conducted under natural conditions it did not indicate the diagnostic efficacy of IRT for mastitis. This comparison was made by Polat et al. (2010) who reported that IRT measurement of udder surface temperature had a sensitivity of 95.6% and a specificity of 93.6% when compared to CMT as the diagnostic standard. They also noted that environmental conditions and the individual characteristics of dairy cows may affect the efficacy of the technique and were "*yet to be evaluated*", despite the fact that this relationship had been reported seven years previously (Berry et al., 2003). Thus, studies supporting the use of IRT for diagnosis of mastitis have shown a response in udder surface temperature to stimulation with LPS, correlation with CMT and diagnostic value compared to CMT.

Another example of the application of multiple temperature measurements by IRT on individual animals is the early detection of febrile disease in beef cattle. In an induction model in which beef cattle were infected with bovine viral diarrhea (BVD), Schaefer et al. (2004) showed that the cannus of the eye provided the most diagnostically relevant temperature measurements compared to other anatomical locations, including the nose, ears, hoof, and the lateral and dorsal surfaces (Figure 2). The cannus temperature was shown to indicate febrile disease 4 to 5 days post-inoculation with BVD. Clinical symptoms were evident 8 – 9 days post-inoculation. Thus, measurement of cannus temperature by IRT had the potential to indicate febrile disease approximately 4-5 days prior to the onset of clinical symptoms. These experiments were conducted under strictly controlled environmental conditions. It would therefore be expected that IRT would not be as sensitive in a real-world setting due to confounding factors such as changeable environmental conditions. Later experiments utilized a spontaneous induction model for bovine respiratory disease (BRD) complex, in which calves were exposed to typical practices in the beef cattle industry of North America that are known to be high risk factors for transmission of BRD (Schaefer at al., 2007). These included exposure to other cattle at auction, during transport and mixing with unfamiliar cohorts in a feedlot pen. An animal was deemed to be true positive for BRD if it exhibited two or more of the following criteria:

- A core (rectal) temperature ≤40°C
- A white blood cell (WBC) count <7 or >11 × $10e^{9}.L^{-1}$
- A clinical score ≥3 (NB: based on ratings for digestive, respiratory and disposition criteria)
- A neutrophil to lymphocyte (N/L) ratio <0.1 or >0.8

In this model, a hand-held thermal camera was used to take IRT images from a fixed distance of the canthus of the eye of calves on arrival at the Lacombe Research Centre (Alberta, Canada) and at weekly intervals until the end of the study. Thermal images were also taken if animals were treated for illness at any time during the study. In this study, eye temperature was shown to predict sick animals 4 – 6 days prior to clinical assessments. However, for this technology to be of practical value for predicting illness in feedlot cattle it was necessary to automate the processes of image capture, temperature measurement and algorithmic analysis of temperature data for diagnostic predictively. To this end an automated, thermal image capture station was constructed based around the water system in a feedlot pen. A photograph of the station is shown in Figure 4 (Schaefer et al., 2012). Calves were fitted with RFID tags that identified individual animals and triggered the system to move an IRT camera to one of two positions depending on the calf's location. The system automatically recorded images of the eye region, obtained relevant temperature data from captured images, and stored temperature information in a database. The test referent can be a comparison of the individual animal's temperature to the mean temperature of its cohorts in the same pen, or each animal can be its own control by comparison of its current temperature with previous recordings. In a small group of animals (n = 65) with a BRD prevalence of 14% the system demonstrated the potential to predict febrile disease approximately 2 days prior to the onset of clinical symptoms (Schaefer et al., 2012). There is much work still to be done with this type of surveillance, not the least of which is a more in-depth analysis of the diagnostic predictability of the various test referents that are achievable using an automated, multi-sampling system.

A similar system has been established in swine barn at Lacombe Research Centre (Lacombe, Alberta, Canada) utilizing a commercially available feeding system called Firestart

(Osborne Industries Inc, KS, USA). The Firestart system identifies individual animals by RFID, and records their weight and feed consumption. An IRT camera mounted on the Firestart system is triggered using the RFID reader to take thermal images. This system permits the automated recording of feed consumption, weight and temperature for individual pigs over extended periods of time. Data from these studies have yet to be analyzed in terms of disease detection but it is expected that a combination of these variables will have greater diagnostic utility than any single parameter.

MEASUREMENT OF GROUP TEMPERATURE BY IRT

An automated system for disease detection in individual animals requires sophisticated technologies and software that are able to;

- Identify individual animals
- Trigger a thermal camera to record images
- Decide on the utility of the image, *i.e.* the correct anatomical location
- Obtain temperature measurements from those images
- Compare temperature measurements to reference data.
- Make a decision on diagnostic relevance.

A far less sophisticated system is to capture temperature data on a group of animals, particularly in densely populated housing systems such as swine and poultry barns. This has the advantage of being technologically simpler since it does not rely on identifying individual animals, but the disadvantage is that it would probably be less sensitive in detecting disease outbreaks. Nevertheless, there is some empirical evidence to support the value of such a system.

Friendship et al. (2009) used a hand-held thermal camera to scan groups of penned pigs (N = 20/Pen), during an outbreak of *Actinobacillus pleuropneumoniae.* A thermal scan was obtained of all pens in which at least one case of mortality was recorded and the maximum temperature of the group of animals in that pen compared to the maximum temperature of three other randomly chosen pens that did not exhibit mortality. Temperature data were compared over three days that included the day that a case of mortality was recorded and for the previous two days. It was noted that pens exhibiting cases of mortality had a significantly higher maximum temperature compared to control pens for all days. It was suggested that thermal scanning of pens could provide producers with an early warning of potential disease outbreaks. The researchers also suggested that mounting a camera in a barn and taking still images over a period of time would reduce the input required by the producer. As previous discussed in relation to mass screening, the use of a hand-held thermal camera is prone to variation in temperature measurement due to the inherent inconsistencies in the application. Mounting a camera in a fixed position in the barn would produce much less confounding variation and thus would be more sensitive to thermal aberrations.

A major difficulty in testing these hypotheses is that disease induction models normally require the use of bio-containment facilities. As a substitute, recent experiments conducted at Lacombe Research Centre (Alberta, Canada) have utilized a vaccination model for stimulation of immune function. Groups of piglets (n = 7/group) were housed in individual pens with an IRT camera mounted on the ceiling directly above the pen. Treatment groups consisted of an untreated (Control) group, a saline injected group (Sham) and a vaccination group (Vac). The vaccination consisted of an intramuscular injection of Farrowsure Gold B. This is a 3-way vaccination for porcine parvovirus (PPV), erysipelas caused by *Erysipelothrix rhusiopathiae,* and leptospirosis caused by *Leptospira bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and *L. pomona.* Thermal images were collected at 5-minute intervals for a minimum period of 3 days beginning one day prior to treatment and extending for 2 days post treatment. Temperature variables included the average and maximum temperatures of the pigs. The mean of 11 replications for the maximum image temperature are shown in Figures 5 and 6. The data are presented as the moving average for 12 images over 24-hour periods synchronized to Time 0 as the point of injection either of vaccine (Vac) or saline (Sham). Note that images were recorded every 5 minutes and thus the moving average represents the mean temperature over the previous hour. The differences in area under the curve (AUC) between the Vac groups *vs.* the Con and Sham groups were significant (Figure 5), but the largest difference was observed in the Vac group between pre- and post-vaccination days (Figure 6). This provides evidence that a thermal response to immunological challenge is evident from images of groups of animals, and probably the best discriminator is to use the group as its own control by comparisons to previous temperature data.

Another potential application for the measurement of group temperature is in poultry flocks. The primary role of feathers is insulation and loss of feathers is very easily detected by measuring radiated heat (Figure 7) (Cook et al., 2006). Note that the conventional method of feather scoring (FS) is a linear scale of 1 - 4 but the relationship of temperature measurements to the FS scale is quadratic. Thus, measurement of temperature by IRT gives a much more accurate assessment of feather cover. Loss of feathers can be due to natural moulting, or can reflect problems associated with nutrition, environmental and social conditions, or disease. Feather losses can have important economic implications in terms of increased feed consumption and are a direct measure of the welfare of birds irrespective of the reasons for feather losses. The remedy for feather loss usually involves changes to flock management, and thus early detection of the problem by IRT could be advantageous in dealing with causative issues. An important disease issue for poultry flocks is infectious laryngeal tracheitis (ILT). In an induction model Schaefer et al. (2008) showed that birds infected with the causative virus exhibited a bi-phasic temperature response of the tympanic membrane. An initial reduction in temperature occurred approximately 1-4 days post-induction (DPI) and a subsequent rise in temperature occurred approximately 9 DPI, corresponding to the appearance of clinical symptoms. There are no examples of this type of surveillance currently in practice but what data is available suggests that IRT has potential as a warning system for ILT infections in poultry flocks.

The use of IRT for disease surveillance is very much still in its infancy. Yet, the potential of IRT as an automated system of disease surveillance in humans and animals is well recognized. The technology for mass screening of individuals with single time-point measurements is probably best utilized as one tool among several, the combinations being of greater diagnostic efficacy that IRT alone. Probably, the greatest potential for IRT in animal industries is in situations that allow for multiple measurements on individuals or groups, and this is most likely to occur in situations involving intensively housed livestock.

REFERENCES

1) Backhans, A. and Fellstrom, C. Rodents on pig and chicken farms - a potential threat to human and animal health. Infection Ecology and Epidemiology 2:1 - 9. 2012.
2) Berry, R. J., Kennedy, A. D., Scott, S. L., Kyle, B. L. and Schaefer, A. L. Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis detection. Canadian Journal of Animal Science 83(4):687-693. 2003.
3) Bitar, D. International travels and fever screening during epidemics: A literature review on the effectiveness and potential use of non-contact infrared thermometers. Eurosurveillance 14(6):1 - 5. 2009.

4) Chiang, M. F., Lin, P. W., Lin, L. F., Chiou, H. Y., Chien, C. W., Chu, S. F. and Chiu, W. T. Mass screening of suspected febrile patients with remote-sensing infrared thermography: Alarm temperature and optimal distance. Journal of the Formosan Medical Association 107(12):937-944. 2008.
5) Chiu, W. T., Lin, P. W., Chiou, H. Y., Lee, W. S., Lee, C. N., Yang, Y. Y., Lee, H. M., Hsieh, M. S., Hu, C. J., Ho, Y. S. and others. Infrared thermography to mass-screen suspected Sars patiens with fever. Asia-Pacific Journal of Public Health 17(26):25 - 28. 2005.
6) Colak, A., Polat, B., Okumus, Z., Kaya, M., Yanmaz, L. E. and Hayirli, A. Early detection of mastitis using infrared thermography in dairy cows. Journal of Dairy Science 91(11):4244-4248. 2008.
7) Cook, N. J., Smykot, A. B., Holm, D. E., Fasenko, G. and Church, J. Assessing feather cover of laying hens by infrared thermography. Journal of Applied Poultry Science 15:274-279. 2006.
8) Friendship, R., Poljak, Z. and McIntosh, K. Use of infrared thermography for early detection of disease causing sudden death in a swine finishing barn. Pages I27 - I28, 28th Annual Centralia Swine Research Update. CentraliaSwineResearch.ca, Ontario. 2009.
9) Hillerton, J. E. Another report on the search for the holy grail - finding the mastitic quarter reliably. Proc. British Mastitis Conference 2003, Newbury, UK. 2003.
10) Hovinen, M., Siivonen, J., Taponen, S., Hanninen, L., Pastell, M., Aisla, A. M. and Pyorala, S. Detection of clinical mastitis with the help of a thermal camera. Journal of Dairy Science 91(12):4592-4598. 2008.
11) Johnson, S. R., Rao, S., Hussey, S. B., Morley, P. S. and Traub-Dargatz, J. L. Thermographic eye temperature as an index to body temperature in ponies. Journal of Equine Veterinary Science 31(2):63-66. 2011.
12) Kunc, R., Knizkova, I., Prikryl, M. and Maloun, J. Infrared thermography as a tool to study the milking process: a review. Agricultura Tropica et Subtropica 40(1):29-32. 2007.
13) Lahiri, B. B., Bagavathiappan, S., Jayakumar, T. and Philip, J. Medical applications of infrared thermography: A review. Infrared Physics and Technology 55(4):221-235. 2012.
14) Lutz, J., Molla, B., Silveira, F., Gebreyes, W. and Lutz, E. A. Using infrared thermal imaging for mass screening of production animals for early detection of febrile diseases. Epidemio et sante anim 59-60:169 - 170. 2011.
15) Ng, E. Y. K. Is thermal scanner losing its bite in mass screening of fever due to SARS? Medical Physics 32(1):93 - 97. 2005.
16) Ng, E. Y. K., Kaw, G. J. L. and Chang, W. M. Analysis of IR thermal imager for mass blind fever screening. Microvascular Research 68:104 - 109. 2004.
17) Nguyen, A. V., Cohen, N. J., Lipman, H., Brown, C. M., Molinari, N. A., Jackson, W. L., Kirking, H., Szymanowski, P., Wilson, T. W., Salhi, B. A. and others. Comparison of 3 infrared thermal detection systems and self-report for mass fever screening. Emerging Infectious Diseases 16(11):1710 - 1717. 2010.
18) Nishiura, H. and Kamiya, K. Fever screening during the influenza (H1N1-2009) pandemic at Narita International Airport, Japan. BMC Infectious Diseases 11:1 – 11. 2011.
19) Pascoe, D. D. and Fisher, G. Comparison of core and skin surface measuring sites for the assessment of body temperature. Thermology International 19(1):35 - 42. 2010.
20) Pascoe, D. D., Ring, E. F., Mercer, J. B., Snell, J., Osborn, D. and Hedley-Whyte, J. International standards for pandemic screening using infrared thermography. Proc. Medical Imaging: Biomedical Applications in Molecular, Structural, and Functional Imaging. 2010.
21) Pezeshki, A., Stordeur, P., Wallemacq, H., Schynts, F., Stevens, M., Boutet, P., Peelman, L. J., De Spiegeleer, B., Duchateau, L., Bureau, F. and others. Variation of inflammatory dynamics and mediators in primiparous cows after intramammary challenge with Escherichia coli. Veterinary Research 42(1). 2011.

22) Polat, B., Colak, A., Cengiz, M., Yanmaz, L. E., Oral, H., Bastan, A., Kaya, S. and Hayirli, A. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. Journal of Dairy Science 93(8):3525-3532. 2010.
23) Ring, E. F. J., Jung, A., Zuber, J., Rutowski, P., Kalicki, B. and Bajwa, U. Detecting fever in Polish children by infrared thermography. 9th International Conference on Quantitative InfraRed Thermography, Krakow, Poland. 2008.
24) Sakaguchi, J., Tsunoda, M., Wada, K., Ohta, H., Kawashima, M., Yoshino, Y. and Aizawa, Y. Assessment of border control measures and community containment measures used in Japan during the early stages of pandemic (H1N1) 2009. PLoS One 7(2):1 - 9. 2012.
25) Schaefer, A., Church, J. S., King, R., Cook, N., Annet, C., Lepage, P., Colyn, J., Marchand, S. and Holt-Klimek, L. Non-invasive detection of infectious laryngotracheitis (ILT) in poultry using infrared thermography. Canadian Journal of Animal Science 88(1):138. 2008.
26) Schaefer, A. L., Cook, N., Tessaro, S. V., Deregt, D., Desroches, G., Dubeski, P. L., Tong, A. K. W. and Godson, D. L. Early detection and prediction of infection using infrared thermography. Canadian Journal of Animal Science 84(1):73-80. 2004.
27) Schaefer, A. L., Cook, N. J., Church, J. S., Basarab, J., Perry, B. J., Miller, C. and Tong, A. K. W. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. Research in Veterinary Science 83:376 - 384. 2007.
28) Schaefer, A. L., Cook, N. J., Bench, C., Chabot, J. B., Colyn, J., Liu, T., Okine, E. K., Stewart, M. and Webster, J. R. The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. Research in Veterinary Science 93:928 - 935. 2012.
29) Scott, S. L., Schaefer, A. L., Tong, A. K. W. and Lacasse, P. Use of infrared thermography for early detection of mastitis in cows. Proc. Proceedings of the Agricultural Institute of Canada, Agri-Food 2000, Winnipeg. 2000.
30) Viguier, C., Arora, S., Gilmartin, N., Welbeck, K. and O'Kennedy, R. Mastitis detection: current trends and future perspectives. Trends in Biotechnology 27(8):486-493. 2009.
31) Witmer, G., Fine, A. E., Gionfriddo, J., Pipas, M., Shively, K., Piccolo, K. and Burke, P. Epizootiologic survey of Mycobacterium bovis in wildlife and farm environments in northern Michigan. Journal of wildlife diseases 46(2):368-378. 2010.

Figure 1: Infrared image capturing mice in the feeder of a pig pen.

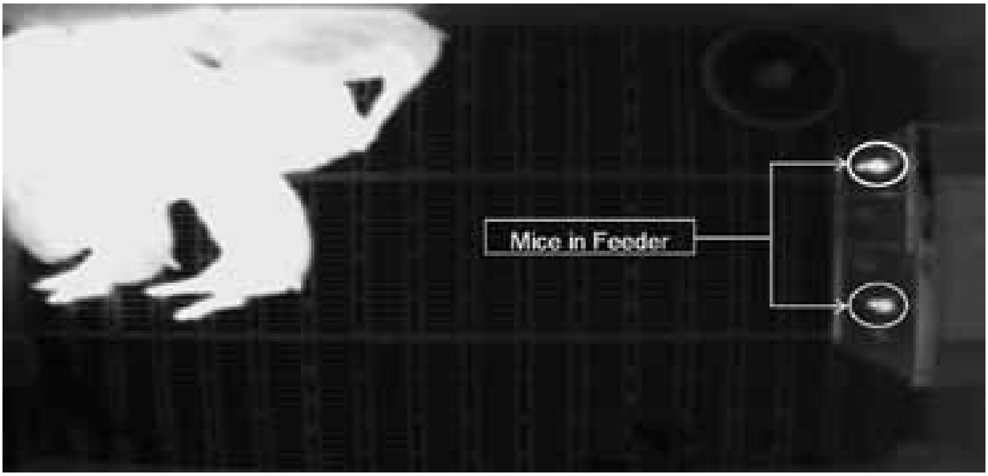


Figure 2: Infrared images of the eye region of cattle following induction of bovine viral diarrhoea and showing increase in maximum image temperature in days post induction.

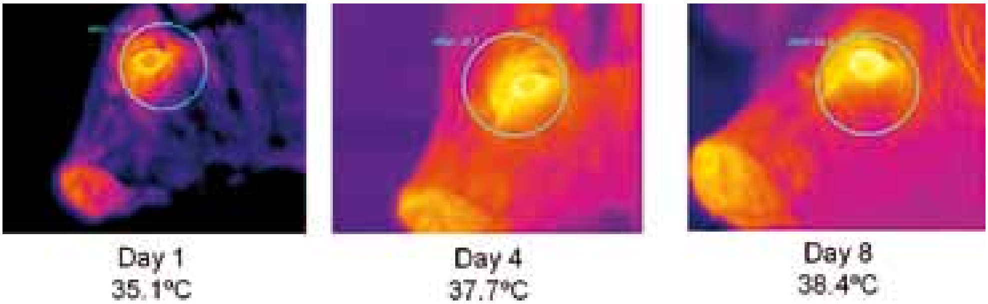


Figure 3: Infrared images of the udder of a dairy cow 1 hour before and 1-6 hours after injection of E. coli lipopollysacharide. Temperature parameters were the maximum and average temperature of a trace of the whole udder surface.

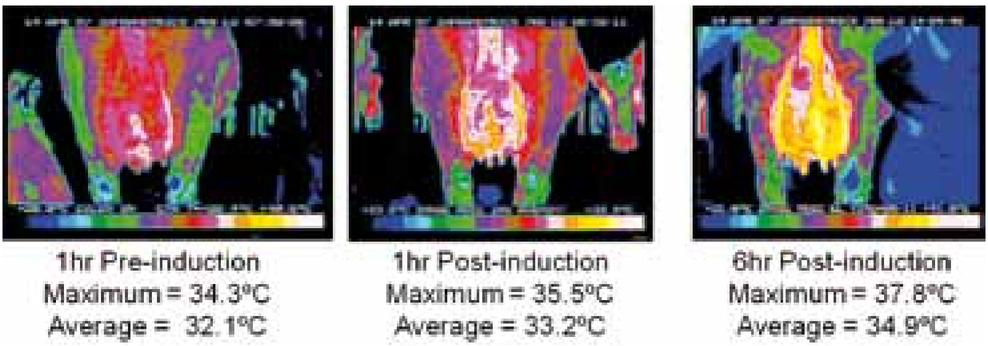


Figure 4: Automated thermal image and analysis capture station in a beef feedlot pen.

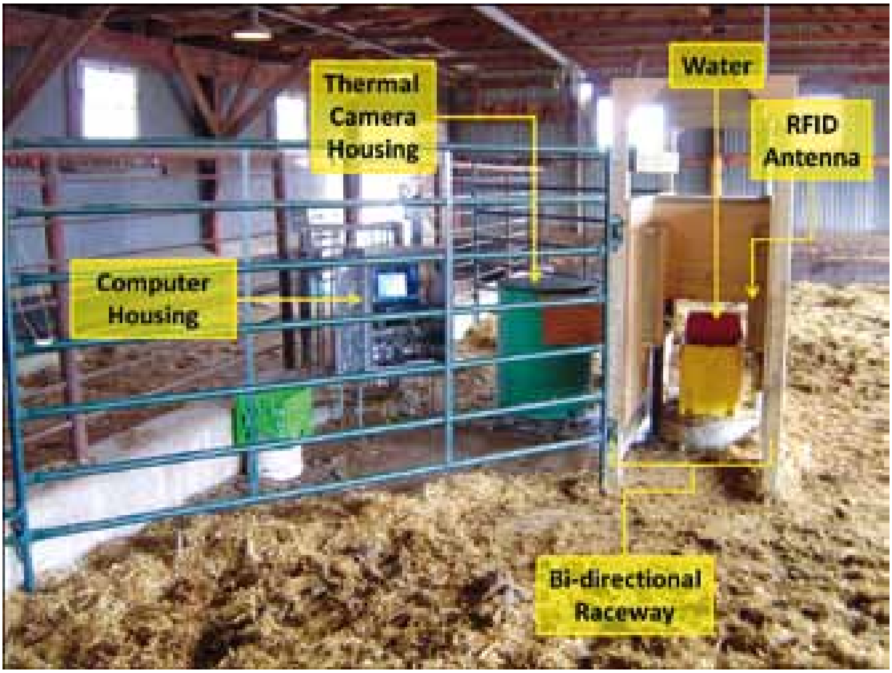


Figure 5: Running hourly average of maximum group image temperatures of Control, Sham and Vaccinated weaned piglets.

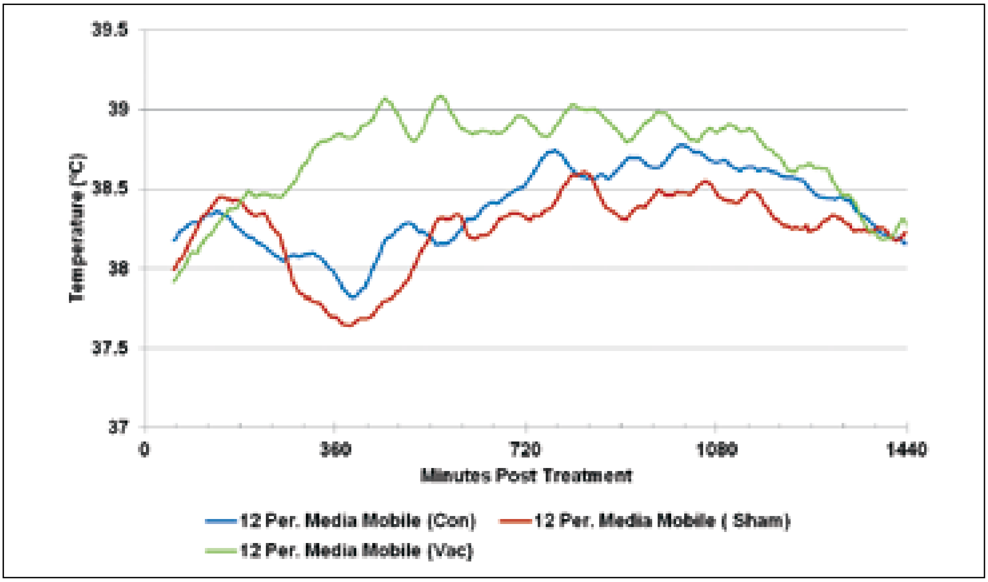


Figure 6: Running hourly average of maximum group image temperature of weaned piglets before and after vaccination.

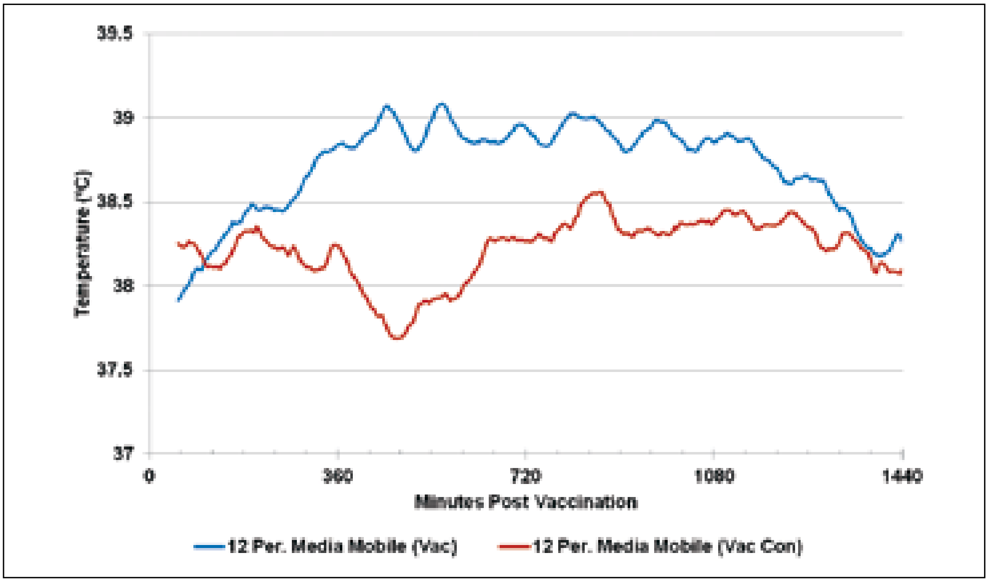
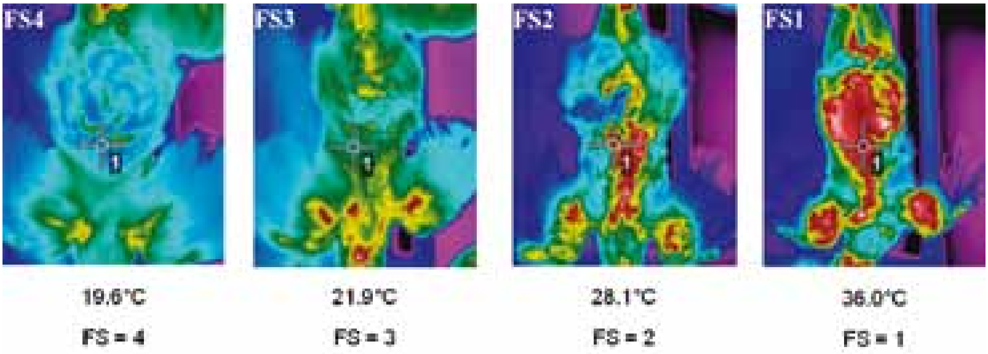


Figure 7: Infrared images of laying hens exhibited various degrees of feather losses and the relationship between subjective scoring and temperature measurements.



INFRARED THERMOGRAPHY IN EQUINE AND CATTLE

EMANUELA VALLE

University of Torino, Italy

ABSTRACT

Infrared thermography (IR) can help the practitioner to identify certain diseases that affect the locomotors system of the horse for different reasons: it has the potential vantage to produce real time images and could help to identify the temperature changes of the anatomical site. Thermography facilitates the localization of increased heat production due for example to inflammation or injury, or a decreased heat production due to reduced blood flow or vasomotor tone. In particular with inflammation is it possible identify an hot spot ; if there is 1°C of differences between two anatomically symmetric regions this could indicate the presence of inflammation. For this reason IR could be applied to many different orthopedic problem of the horse like foot disease as laminitis, chronic palmar foot, abscesses and corn and foot imbalance. Also early sign of joint inflammation can reportedly be detected before lameness resulting from osteoarthritis.

In the case of tendon inflammation a hot spot could be seen over the site of inflammation before physical evidence of swelling or pain. Ligament injuries such as suspensory desmitis can also produce hot spot. IT could be useful to identify dorsal metacarpal disease or fractures on radius and tibia. Also inflammation associated to muscle generate hot spot on the IT imagines. Influential tissue changes may be detected as much as 2 week before the onset of the clinical sign with obvious benefit since the camera is at least 10 times more sensitive than clinician's hand.

Key words

Thermography, inflammation, orthopedic problem, foot disease, ligament injuries.

INTRODUCTION

Infrared thermography (IT) has been used in equine orthopaedics for a number of years (Bathe, 2011). It can help the practitioner to identify certain diseases that affect the locomotor system of the horse for different reasons: it has the potential advantage to produce real time images and could help to identify the temperature changes of the anatomical site. In fact, local circulation and relative body flow determine the thermal pattern of each anatomical region (Yanmaz et al., 2007).

Thermography does not reveal exact pathologies, but facilitates the localization of increased heat production due, for example, to inflammation or injury, or a decreased heat production due to reduced blood flow or vasomotor tone (Eddy et al., 2001).

With inflammation, one of the most important signs is the production of heat. It is an important sign of inflammation and has been shown to be relatively effective in the assessment of the inflammatory process in the horse (Turner et al., 2001). Therefore with inflammation is it possible to identify a *"hot spot"* point on the skin directly above the underling injury (Turner, 2011). If there is 1°C temperature difference between two anatomically symmetrical regions, this could indicate the presence of inflammation (Yanmaz et al., 2007). The normal temperature range of human, non-injured athletes showed a side-to-side variation of 0.3 °C

(±0.61°C) (Hildebrandt et al., 2010). IR can detect temperature changes before such changes could be detected by palpation. This is beneficial for the diagnosis of the lesions that could induce lameness in horses.

Influential tissue changes may be detected as much as 2 weeks before the onset of the clinical signs with obvious benefit (Simon et al., 2006). The camera is at least 10 times more sensitive than clinician's hand (Turner et al., 2001).

HOW TO PERFORM IR THERMOGRAPHY IN THE HORSE

To take advantage of IT it is necessary to have good imaging acquisition to avoid artefacts and confounding factors. For this reason it is useful to obtain images under standardized conditions.

- Ideally thermography should be performed in a room with a temperature range between 20-25°C. (Simon et al., 2006). However according to Yanmaz et al., 2007, 17°C is still appropriate for IT. In colder weather, it can be advantageous to have a warmer location for the thermal imaging. Standardizing the ambient temperature is necessary since the distal aspect of the limb in particular has an important thermoregulatory role. Blood supply can be reduced dramatically to conserve heat in cold conditions (Bathe, 2011). It is also important that the horse is acclimatized for 10-20 minutes in the environment where the imaging acquisition will be performed. IT images should be taken in a relative bare room without radiant heat sources, draft or sunlight (Bathe, 2011). Darkness or low level lighting is the ideal condition to perform the examination in a room where the airflow is uniform to avoid erroneous cooling (Yanmaz at al., 2007)
- The horse should be at rest and needs to stay calm and quiet during the examination. If the horse has been heavily exercised then a 2 hour period should be allowed for recovery before imaging acquisition. According to Turner et al. (2001) feet of horses continued to be hot for almost 24 h after a maximal exercise at gallop. Physical exercise results in heat production since only 20-25 % of the energy produced is converted into mechanical energy for muscle contraction (Simon et al., 2006). The remaining part is lost by the body as heat. Sedatives should not be used during the examination as they could affect peripheral circulation, cardiovascular system (Yanmaz at al., 2007) and promote sweating. Imaging could be done after the horse has been trotted or lunged with the objective of increasing the blood supply to the limbs and so increasing the contrast of the foot relative to the background (Bathe, 2011).
- Generally the horse should not clipped; if the hair coat is very long it could acts has insulator reducing the contrast of the imagine. Hairless or clipped regions will appear warmer because hair insulation is not present (Eddy et al., 2001). The coat should also be clean and dry and the feet need to be cleaned to remove dirt.
- No bandages or boots should be applied before the examination at least twenty minutes before (Bathe, 2011), until to 2 h (Yanmaz at al., 2007). Liniments or other topical applications should not be applied and acupuncture in the region of examination should not be performed in the week beforehand (Eddy et al., 2001).
- Generally multiple images should be collected of the area under investigation from at least in two directions approximately 90° apart to detect if the hot spot is consistently present (Yanmaz at al., 2007). The neck region should be imaged from the side; the back and the hindquarter should also be imaged not only from the lateral point of view, but also from the dorsal aspect if possible. For the distal part of the limb the images should be performed in four directions: dorsal, palmar or plantar direction, left and right sight (Bathe, 2011). If it is necessary to obtain images of the solar aspect of the foot, the limbs

should be lifted. For the proximal part of the limb images should be taken from the cranial and later side. It is advisable to perform repeated scans of the same suspect area to assure reproducibility and to obtain more reliable information; two to three scans should be performed at least one minute apart (Eddy et al., 2001). The contralateral limb should be generally imaged for comparison symmetry evaluation. However thermoregulatory cutoff could change; there could be discontinuous periods of vasodilatation not essentially symmetrical between limbs. In this situation is important to perform a reassessment of the horse some hours later.

THE NORMAL THERMAL PATTERN OF THE HORSE BODY

The thermal pattern is determined by local circulation and relative blood flow in the area. Based on this fact, some simplification can be made regarding the thermal pattern of the horse. The heat usually follows the course of blood vessels (Bathe, 2011), even if in general veins are warmer than arteries because they are draining blood from metabolically active areas (Yanmaz at al., 2007).

In an examination of the normal distal limb the coronary band is the warmest part of the picture since there is the arteriovenus plexus of the coronary band (Yanmaz at al., 2007). Also, physiological adjustment could occur in the temperature of the coronary band such as an increase immediately after feeding (Redaelli et al., 2011). The hoof wall becomes gradually cooler as it approaches the toe (Bathe, 2011). In the solar aspect the collateral groove of the frog appear as a warmest area and the area between bulbs of the heel appear warmer than the areas close to them. The dorsal part of the distal limb, the metacarpus, the pastern and the fetlock region appear relatively cold since there are fewer major blood vessels in this part of the foot (Turner, 2011).

In the region of the metacarpus there is warmer area between the third metacarpus and flexor tendons due to the median palmar vein in the forelegs and the metatarsal vein in the hindlimb (Yanmaz at al., 2007).

Tendons, and in particular flexor tendons, are seen bilaterally symmetric and consist of elliptical isothermic zones in the thermogram (Turner, 2011). The lowest temperature is located in the centre over the palmar aspect: the peripheral areas near the carpus and fetlock are approximately 1°C cooler(Bathe, 2011).

Generally the ventral midline, the back, the chest and the area between the hindlimb are warmer (Yanmaz at al., 2007) and joints tend to be cooler than the surrounding tissue except when a superficial vessel is present which passes over the articulation (Bathe, 2011). For example along the medial aspect of the hock has a hot spot due to the presence of saphaneus vein (Turner, 2011).

THE PATHOLOGICAL CONDITIONS

IT can be helpful to optimize the efficiency of a lameness evaluation since, as a diagnostic tool, differences of 1°C between two symmetrical regions indicates inflammation. Also a decrease in temperature (*cold spot*) is important. For this reason thermography could be applied as a physiological imaging method in the detection or prevention of injuries especially during training (Turner, 2011).

The foot

The foot region is one of the more useful areas for clinical imaging (Bathe, 2011). Even if superficial foot inflammation can be apparently evaluated without IT, the real advantage of this technique is in the case of a suspected deep pathology; horses with mild signs of deep inflammation or with positive results at hoof testers are the best candidate. IT could also be useful to identify the disease in the early phase or when radiographic findings are questionable. IT can be used in the case of numerous problem of the foot such as laminitis, navicular disease, abscesses and corn (Eddy et al., 2001).

With laminitis there is a loss of the normal temperature pattern since the coronary band is usually warmer by 1-2°C than the remaining hoof (Yanmaz at al., 2007). In this condition there is an increase of the temperature of the wall and the heat appears equally distributed. Also on the solar images there is an increase of temperature especially in the region of the tip of the distal phalanx (Bathe, 2011). IT can be useful in detecting laminitis before clinical signs appear especially for monitoring horses at high risk of becoming laminitic (Eddy et al., 2001). During the developmental phase there changes in the thermographic images without clinical sign. In chronic laminitis there may be areas of decreased temperature in the dorsal aspect of the coronary band which, in general, could be a poor prognostic indicator (Bathe, 2011).

Subsolar or coronary band infection generally results in an increase in temperature such as with corns and subsolar bruising (Bathe, 2011), however in some situations an abscess, before draining, could appear as a relatively cold area. This is the consequence of the pressure caused by the abscess that decreases the blood circulation in the area where the infection is localized (Turner, 2011).

Horses with a chronic palmar foot, in contrast with other inflammatory conditions, are characterized by a reduction in blood flow with a decrease in temperature (Eddy et al., 2001); they could have a normal imaging pattern or a cooler pattern in the heel region. The normal horse sustains a 0.5°C increase in temperature of the foot after exercise. By contrast, 40% of horses with palmar foot pain syndrome do not have an increase (Turner, 2011) which may be due to the decreased load in the area rather than an ischemic disease (Bathe, 2011).

IT could be also helpful in identifying foot imbalance. If a shod horse is trotted on a hard surface and imaged immediately, the side with the major load appears warmer. With a substantial medio-lateral imbalance the lateral wall lands first and rolls over to the medial side during the weight bearing phase, thus increasing the temperature of the medial aspect of the coronary band, rather than on the side that lands first (Bathe, 2011).

The joints

Acute inflammation can occasionally give an increase in the heat pattern, but chronic pathologies are not normally detectable. Early signs of joint inflammation can reportedly be detected two weeks before lameness resulting from osteoarthritis. This is useful to allow modification of training regimes and workload to decrease the risk of a more serious pathology (Bathe, 2011).

When inflammation occurs, an large oval area horizontally medial to lateral (Turner, 2011), is present due to the increased temperature. This area is centred on the joints which is best viewed using imaging from the dorsal aspect (Yanmaz at al., 2007). In the case of the inflammation in the distal limb's joints, the area on the IT images has a circular pattern. No specific correlation could be made between heat and joint damage since it depends from the chronic nature of the problem, the involvement of the synovial component, the circulation and the presence of osteochondral fragments (Turner, 2011).

The tendons and ligaments

In the case of tendon inflammation, a hot spot could be seen over the site of inflammation until two weeks before physical evidence of swelling or pain (Yanmaz at al., 2007). IT has an enhanced ability in detecting lesions in the tendon and this could be useful in preventing further damage by adapting training regimes or modulating the reintroduction of horses with recurring tendonitis back to work (Bathe, 2011).

Ligament injuries such as suspensory desmitis can also produce hot spots (Eddy et al., 2001). IT images of normal suspensory ligaments should be bilaterally symmetric, where the warm regions are usually the medial and lateral aspects due to the location of the palmar vessels. The palmar area normally has the lowest temperature since it is farther from superficial vessels. According to Van Hoogmoed and Snyder (2002) lateral views more accurately detected differences between the limbs compared to the palmar view. This is because inflammation of the suspensory ligament needs to be severe enough to reflect through the flexor tendons.

The long bones

The majority of the long bones are covered by muscle and could not be imaged thermographically (Bathe, 2011). However, the application of IT could be useful in identifying dorsal metacarpal disease or fractures of the radius and tibia. Dorsal metacarpal disease is common in racehorses (Yanmaz at al., 2007), and divided into three grades according to the severity of the disease. IT could help to distinguish grade 3 lesions sooner than a radiograph. Grade 2 and 3 lesions could not be easily distinguishable and the evidence of stress fracture could not be possible for two or three weeks. Grade 1 and 2 are described by a hot spot midshaft over the dorsal cannon bone. In grade 2 lesions the hot spot is not central and is typically observed on the lateral and medial images in addition to the dorsal view. These areas are 2-3°C warmer than the nearby tissue (Turner, 2011).

The muscles

Inflammation associated with muscle generates hot spots on the IT images. Where oedema and swelling are present in the affected muscle, this can inhibit blood flow through the muscle generating a cold spot (Yanmaz at al., 2007). IT could offer important information on muscle injury because it can be used to locate the area of inflammation in the muscle or muscle group and can illustrate the atrophy before it becomes apparent (Turner, 1996).

The most important cause of muscle inflammation is muscle strain, but it is important to verify a consistent left right asymmetry (Bathe, 2011). Comparative images should be practically identical. After areas of inflammation have been examined IT can be used to observe progress in the recovery period (Turner, 1996).

REFERENCES

1) Bathe A. P. (2011) Thermography: Use in Equine Lameness. In Diagnosis and management of lameness in the horse edited by Ross M. W., Dyson S. J. 266-269.
2) Yanmaz L.E., Zafer O. and Dogan E. (2007) Instrumentation of thermography and its Applications in Horses. Journal of Animal and Veterinary Advances, **6**, 858-862.
3) Eddy A.L., Van Hoogmoed L.M. and Snyder J.R. (2001) The role of thermography in the management of equine lameness. The Veterinary Journal, **162**, 172-181.
4) Turner T.A., Pansch J. and Wilson J.H. (2001) Thermographic assessment of racing thoroughbreds. In AAEP Proceedings, **,** 344-346.

5) Turner A. (2011) Thermography. Adams and Stashak's Lameness in Horses, 6th Edition. 1272 pp.
6) Hildebrandt C., Raschner C. and Ammer K. (2010) An Overview of Recent Application of Medical Infrared Thermography in Sports Medicine in Austria. Sensors, **10**, 4700-4715.
7) Simon E.L., Gaughan E.M., Epp T. and Spire M. (2006). Influence of exercise on thermographically determined surface temperatures of thoracic and pelvic limbs in horses. Journal of the American Veterinary Medical Association, **229**, 1940-1944.
8) V., Redaelli, E., Valle, D., Bergero, M., Verga, M., Papa, F. Luzi (2011). Infrared thermography used to measure physiological variation of coronary band temperatures in horses after feeding. In Nutrimi Conference, Rho fiera, 7 aprile.
9) Van Hoogmoed, L.M. and Snyder J.R. (2002). Use of infrared thermography to detect injections and palmar digital neurectomy in horses. Veterinary Journal, **164**, 129-141.
10) Turner T. (1996). Thermography as aid in the localization of upper hindlimb lameness. Pferdehelkunde, **12**, 632-634.

Figure 1: Normal thermographic images of the horse body. The heat track the course of blood vessels. The hoof becomes gradually cooler as it approaches the toe.

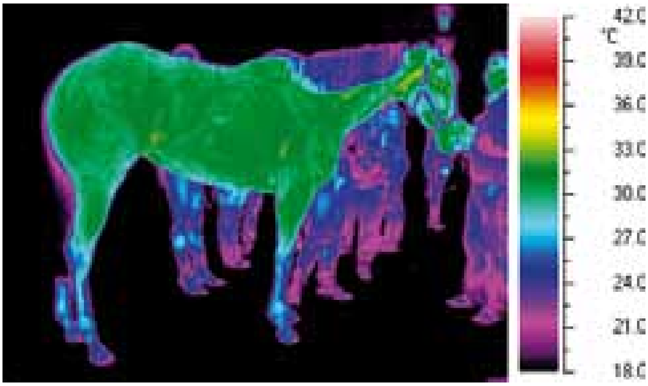
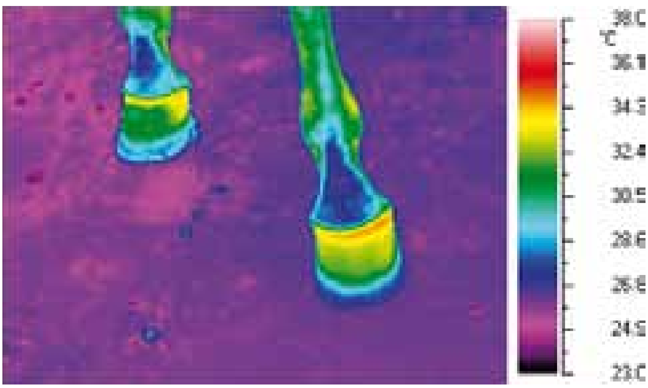


Figure 2: Thermographic images of the foot. The left coronary band shows an increase of the temperatures due to inflammation.



INFRARED THERMOGRAPHY IN EQUINE AND CATTLE

STEFANO TARANTINO

DVM free practitioner, Milano, Italy

ABSTRACT

Infrared Thermography is a non invasive method inspecting heat variations coming from digital regions. It can be used in order to inspect inflammations. An experimental pattern in monitoring lameness outbreaks is proposed. Control strategies connected to early diagnosis can be obtained.

Key words

Thermography, dairy cows, lameness, diagnosis, infrared thermography, not invasive method.

INFRARED THERMOGRAPHY IN CATTLE

Infrared Thermography (IRT) deals with image diagnosis. The absence of undesirable effects (no radiation, no need for pharmacological or physical restraint, no flash photography and, most of all, no contact with the animals) means that it can be used in the field diagnosis of large animals.

Whenever measurement of body temperature might be fundamental in formulating a diagnosis, IRT may theoretically be used successfully. It is ideal when working with large numbers of animals to establish diagnostic patterns. This technique gives a "visual" idea of the heat variations on the surface of a body, with good accuracy and sensitivity. With this technique inflammations or circulatory problems may be detected by investigating higher or lower levels of heat emission.

Recent IRT cameras provide sharp images, are easy to handle and strong enough to be suitable for on-farm use. Because the technology is compact and non-contact, it allows, for example, the examination of cows walking along the feeding stalls or standing in the milking parlour. Moreover, a large number of images can be collected, both in "static" or in "dynamic" mode.

Statically derived thermal images are obtained by using the thermal camera as a normal camera, while dynamic images are obtained by freezing frames following a pre-selected time interval. Video recordings can also be made.Environmental conditions play an important role and they must always be considered: wet body surfaces may show lower temperatures and animals under direct sunlight may appear warmer.

Thermography may represent a great help in a dairy veterinarians everyday practice; the best benefit can be achieved by planning long-term monitoring systems. Since the cost of the device is high and it requires a very well trained technician in order to get good images and correctly interpret them, there is no value in using IRT for spot diagnosis.

Environmental factors, such as air temperature, shadows, time of day and seasonal factors can affect the final result and must always be considered carefully.

Only few of the various uses of IRT are presented in this article and this short article is far from being a complete and in-depth investigation.

Some areas of the body produce better thermal images than others. Large muscles or intra-abdominal organs cannot easily be inspected. The udder, for example, gives images difficult to understand for two main reasons, its spherical shape and the amount of fluids it contains.

In the first hypothesis we deal with a maximum volume and a minimal surface solid. In the second, milk, intra cellular fluids and blood work like water, thus generating a great number of heat sources.

Other organs give us very interesting thermal images. Distal limb regions may easily be inspected such as from the carpus (tarsus) to the claw, as shown in Figure 3.

Hoof diseases are one of the most important unresolved problems in the dairy industry all over the world (Greenhough, 2007).

Welfare, milk yield and body weight rapidly decrease during a lameness episode.

The consequences of a digital disease can reduce the animal performance for up to five months after the outbreak of the pathology.

We may divide digital problems into two main parts, infectious (Digital Dermatitis DD, Interdigital Phlegmon PHL) and bio-mechanical (Sole Ulcer SU and White Line Disease WLD) (Greenhough, 2007). Other problems, such as toe/heel abscesses are frequently detected during hoof trimming and are often involved in bovine lameness .

Recent work (3,4) has demonstrated a strong connection between pathology prevention using IRT images and clinical examination performed during hoof trimming with a sensitivity of 93% on hind limbs. Around 95% of hoof problems affect the rear limbs in cows and rear lateral claws are more frequently involved. Differences in localization, depth, tissues, structures and kinds of lesions still exist, while some diseases hit horn or inner structures and others only the skin.

IRT only shows differences in heat and thermal dispersion of one kind of lesion might be different from another. These differences might be significant in evaluating IRT specificity between front and rear limbs.

Currently IRT is being used as a continuous and long term monitoring system, shifting the objective from having a single frame taken on one day to creating a pattern to control claw diseases in cattle all the year round.

The first step of the study, linking IRT with veterinary diagnosis during hoof trimming, with cows restrained in a suitable chute where the cows' limbs were raised off the ground. The second step of the study plans to study dairy cows during milking or in feeding stalls, avoiding contact with the cattle and keeping 3 meters away from the animals.

Criteria under consideration for the farm are:

- the presence of any significant diseases,
- the availability of an up to date database of hoof diseases,
- logistics that allow simple and quick IRT image collection, and
- imposing no stress on the cows.

The current study is working with a 650 Italian Holstein Fresian dairy cow farm, using a rotary milking parlour, which fitted these parameters and where the database is updated daily. .

An IRT camera is securely mounted 3 meters away from the cows to record images of the hind claws. The ID of the cow is acquired at the same time by transponders and the results are compared with the lameness severity assessed by "locomotion score" (Sprecher et al. 1997). Every cow judged to be abnormal is treated by the vet. After this field trial, a strong decrease of SU and WLD was observed. These data still need to be confirmed, but a statistical evaluation will soon be available.

REFERENCES

1) Paul R. Greenhough "Bovine laminitis and lameness. A hands-on approach" Saunders Elsevier 2007.
2) V. Redaelli, S Tarantino, C Ricci, F Luzi, A Zecconi, M Verga (2009) A non invasive method to measure lameness in dairy cow. Ital. J. of Anim. Sci., 8 (Suppl. 2):671.
3) A Zecconi, V Redaelli, S Tarantino, F Luzi, M Verga. (2011). Infrared thermography as a non invasive technique to assess lameness in dairy cows. Conference Lameness in ruminants: lameness, a global perspective. Rotorua (NZ).
4) D.J. Sprecher, D.E. Hostetler, J.B. Kaneene (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology, Volume 47, Issue 6, 15 April 1997, Pages 1179–1187.
5) Hilsberg Clinical application of infra red thermography in inflammation diagnosis in mega herbivores European Association of Zoo and Wildlife Veterinarians (EAZWV) 4th scientific meeting,joint with the annual meeting of the European Wildlife Disease Association (EWDA) May 8 12, 2002, Heidelberg, Germany.
6) K. S Swatrtzkopf-Genswein J. M. Stookey "The use of infrared thermography to assess inflammation associated with hot iron and freeze branding in cattle" Canadian Journal of Animal Science, vol., 77; n°4:577-583.
7) D. J. McCafferty "The value of infrared thermography for research in mammals: previous applications and future directions" Mammal Review, Volume 37, Issue 3:207-223.
8) M. A. Mitchell, P. J. Kettlewell, M. Villaroel-Robinson, M. Farish, E. Harper. "Continuous recording of deep body temperature to assess thermal stress in livestock during road transportation" Proceedings of the British Society of Animal Science and the Agricultural Research Forum2010 Advances in Animal Biosciences: 173.

Figure 1: An example of IRT taken from a Holstein Fresian cow, showing the differences in temperature between the distal region of the right front limb and the other limbs. The heel and the flexor tendons area are involved.

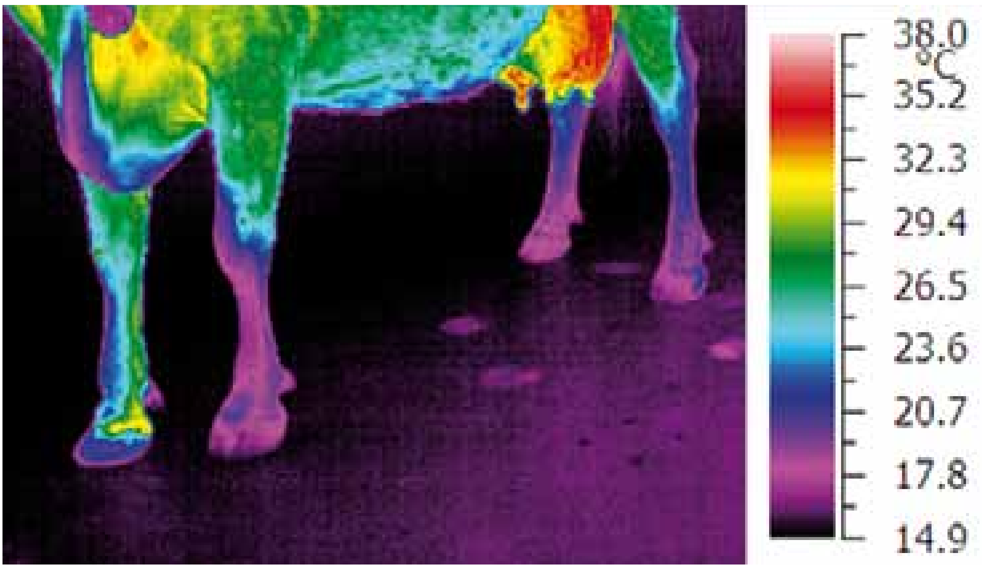


Figure 2: This cow was showing a locomotion score of 3 (severe), indicating a degree of lameness. During the vet/hoof trimmer visit a normal (left) and a IRT (right) image of the same claw was taken. Thermal abnormalities in the lateral heel region are clearly visible. A toe/heel abscess was present.

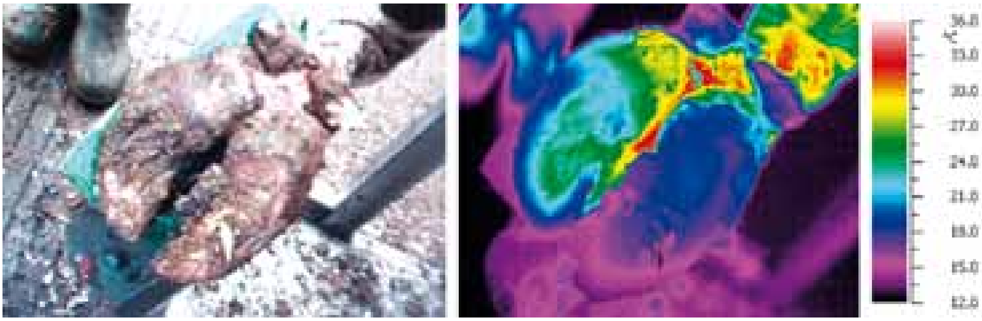
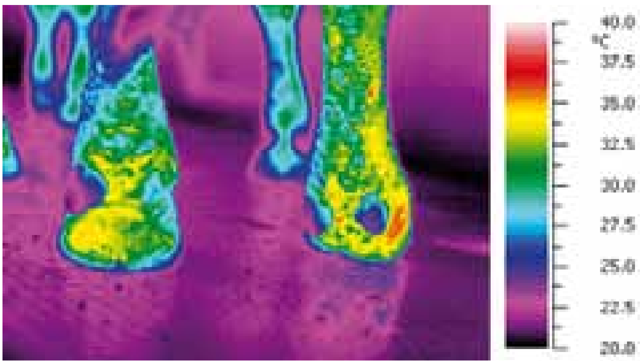


Figure 3: A normal thermal outcome in a sound claw. This cow was showing a locomotion 1 (normal) score during the gait.

Figure 4: A lame cow (locomotion 3) showing a warm area in the digital region. White line disease was suspected.



NEW DEVELOPMENT IN SENSORS TO DETECT MASTITIS IN DAIRY COWS

ALFONSO ZECCONI

DIVET, Università degli Studi di Milano, Italy

ABSTRACT

There is an increasing number of application of new technologies to manage dairy herds and, specifically, of "on line" systems to diagnose diseases or physiologic status. Among the most interesting tools available to detect diseases or abnormal health status of dairy cows, there is infrared thermography (IRT). IRT and other techniques are based on the use of sensors. This latter term gained a widespread use to indicate technologies measuring chemical, physical or visual parameters to detect diseases, physiological status or behavior in dairy farms. Mastitis causes changes in milk constituents by the means of chemical mediators of inflammation, bacterial metabolites and toxins, and the activity of cellular and bacterial enzymes. The changes in milk composition have been used as a target in several sensors for on-line detection of mastitis or abnormal milk. In this area, two of the most appealing applications of IRT are monitoring teat tissue conditions and clinical mastitis diagnosis. The difference in teat temperature measured pre and post milking may indicate that machine milking induces long lasting alterations in teat fluid dynamics, and that these alterations can be assessed by IRT. Besides this latter application, there is an increasing interest to use IRT as a tool to detect mastitis. Indeed, a thermal camera installed in a milking or feeding parlor might be able to detect clinical mastitis with fever before milking avoiding the delivery of milk from diseased cow to the dairy factory. Few studies are available, but their results are not consistent, yet.

Key words

Dairy cows, milk, milk production, milk quality, herd health management, diagnosis, milking machine, sensors, on-line detection, mastitis, infrared thermography, biomarkers, conductivity, superficial temperature.

INTRODUCTION

The current dairy production scenario is characterized by a decreasing number of dairy farms, by an increase of herd size and milk yield, and by a large volatility of milk prices.

This scenario requires several changes in herd management in order to increase efficiency of dairy farms. Among these changes are the increasing application of new technologies to manage dairy herds and, specifically, "on line" systems to diagnose diseases or physiological status (i.e. heat or pregnancy). Several tools have been developed to measure cow activity, moving behavior and physiological changes as tools to monitor cow health and fertility (Brandt et al., 2010; Hogeveen and Ouweltjes, 2003).

Among the most interesting tools available to detect diseases or abnormal health status of dairy cows is infrared thermography (IRT). IRT is a non-invasive thermal profile visualization technique allowing the measurement of changes in temperature at different body sites, including the eye, udder and foot (Alsaaod and Buscher, 2012; Barth, 2000; Rainwater-Lovett et al., 2009; Schaefer et al., 2012; Stokes et al., 2012).

SENSORS

The term “sensor” gained widespread use to indicate technologies measuring chemical, physical or visual parameters to detect diseases, physiological status or behavior in dairy farms, and it will be used in this chapter within this definition.

The rapid development of sensor technologies and potential applications increased the need for an objective evaluation of sensors performance and epidemiological accuracy. An unbiased evaluation and comparison of the different sensors is still unavailable both at the scientific and practical level, even if these problems started to be addressed, for instance, by scientific organizations such as International Dairy Federation. The evaluation and comparison of sensor performances, including IRT, are out of the scope of this chapter. However, it is important to suggest that if a sensor detection system has to be commercialized it should always state the values of four epidemiological characteristics and in which conditions these data were obtained. This will allow an objective evaluation of the new tool and the assessment of potential benefits once applied in the specific herd.

The proposed four epidemiological characteristics are the following:

- **Sensitivity**, defined as the proportion of true positives for the considered parameter (i.e. mastitis, ketosis, heat) that are detected by the sensor.
- **Specificity**, defined as the proportion of true negatives for the considered parameter (i.e. mastitis, ketosis, heat) that are detected by the sensor.
- **Positive predictive value** defined as the proportion of subjects with sensor positive results that are in the status considered (i.e. mastitis, ketosis, heat).
- **Negative predictive value** defined as the proportion of subjects with sensor negative results that are not in the status considered (i.e. mastitis, ketosis, heat).

MASTITIS

Mastitis is defined as an inflammatory disease of mammary gland. It is one the most important production diseases in dairy cattle and is caused by several bacterial species. Mastitis is either clinical or subclinical, but in both cases milk produced has a different composition when compared with milk produced by healthy cows. The changes in milk constituents are caused by chemical mediators of inflammation, bacterial metabolites and toxins, and the activity of cellular and bacterial enzymes. The changes in milk composition concern both soluble and cellular components and they have been used as a target in several sensors for on-line detection of mastitis or abnormal milk (Hogeveen and Ouweltjes, 2003).

Table 1: Milk composition changes at different levels of somatic cell counts (Zecconi et al., 2010).

Component	Somatic cell counts x 1000 / ml			
	<100	<250	500-1.000	> 1.000
	Reduction (g/100 ml)			
Lactose	4.90	4.74	4.60	4.21
Caseins	2.81	2.79	2.65	2.25
Fat	3.74	3.69	3.51	3.13
	Increase (g/100 ml)			
Serum proteins	0.81	0.82	1.10	1.31
Serum albumins	0.02	0.15	0.23	0.35
Immunoglobulins	0.12	0.14	0.26	0.51
Chlorine	0.091	0.096	0.121	0.147
Sodium	0.057	0.062	0.091	0.105
Potassium	0.173	0.180	0.135	0.157
pH	6.6	6.6	6.8	6.9

Subclinical mastitis can be diagnosed only by means of microbiological and cytological analyses, because there are no visible changes in milk or tissues. Clinical mastitis, inducing more or less severe changes in tissues in addition to milk alterations (e.g. clots and flecks) can be diagnosed by visual (clinical) evaluation. However, if the more acute ones are easy to detect visually, the smaller changes in tissue temperature are not usually detected, and they are the potential target of IRT as a means for an early detection of clinical mastitis (Barth, 2000; Hovinen et al., 2008).

It should be emphasized that most of the sensors proposed or applied to mastitis diagnosis are not performing a diagnostic test but they only detect some type of variation of the considered parameter issuing an alarm, thus suggesting which cow should be examined by proper clinical or laboratory diagnostic procedures.

In the following sections, the different sensors applied to mastitis are briefly described with a comparison to the potential features of IRT in the field of mastitis diagnosis.

SENSORS TO DETECT CLINICAL MASTITIS

Color and Image Sensors

In milking hygiene regulations both in Europe and USA, the detection and separation of abnormal milk are mandatory. While this task can be performed visually by the dairyman in conventional milking parlours, with the introduction of automatic milking systems (AMS), which operate without the presence of an operative, sensors have to take over detection of clinical mastitis and abnormal milk.

Different technologies have been proposed, but only two gained a wide application, namely optical/colorimetric measurement and electrical conductivity (EC). Detection of abnormal color can be realized by spectroscopic and colorimetric measurements. Colostrum can be optically differentiated in milk by color variations in the blue region, while milk with a reddish color indicates the presence of blood suggesting the presence of mastitis or of a teat/udder lesion. The optical system commercially available on AMS showed a sensitivity of 68%, but with a positive predictive value of only 32% (Brandt et al., 2010).

ELECTRICAL CONDUCTIVITY

Electrical conductivity is the oldest and most widespread technology available in AMS and on conventional milking machines. Electrical conductivity (EC) is a measure of the resistance of a particular material to an electric current. The ions in milk conduct electricity such that any change in the concentration of ions is reflected as a change in conductivity. Inorganic salts are the main contributors to conductivity with 60% of the total conductivity due to sodium and potassium chloride (Hamann and Zecconi, 1998).

Mastitis leads to a change in blood capillary permeability, resulting in changes in the ion concentrations. This change in EC can be used as an indicator for mastitis, even if other factors such as temperature, the fat content and milk fraction affect the electrical conductivity of the milk.

A meta-analysis of the available literature showed that EC does not identify mastitic quarters or cows with sufficient accuracy, especially for subclinical mastitis detection (Hamann and Zecconi, 1998).

SENSOR TO DETECT CLINICAL/SUBCLINICAL MASTITIS

Somatic cell counter

Milk somatic cells (SC) are the leukocytes normally present in milk and represent a defense mechanism against infections. Their number increases as a consequence of the development of an inflammatory process, when a quarter becomes infected. Somatic cell counts (SCC) are therefore the most-used method to detect mastitis and to assess milk quality worldwide (I.D.F., 1995; N.M.C., 1996). Cell counts are usually performed in milk quality laboratories using cytofluorimetric instruments specifically developed for this task. However, only recently have cell counters that can be used cow-side or on-line become available. These systems apply the same technologies as the laboratory counters and their performances are very similar to the laboratory instruments.

Alternatively, SCC can be assessed by other methods such as near infrared (NIR) technology or by optical methods, both of which are commercially available (Brandt et al., 2010). Even if the accuracy of these tests showed to be relatively high, it is still lower when compared to SC counters applied on milking machines.

L-LACTATE DEHYDROGENASE SENSORS

L-Lactate dehydrogenase (LDH) is an enzyme produced by leukocytes and its concentration in milk has been shown to be related to SCC (Chagunda et al., 2006; Friggens et al., 2007). Sensors measuring LDH have been developed and applied to an on-line milk diagnostic system (Herd Navigator, DeLaval Sweden) in a system which applies a biometric model to issue alarms on different parameters (mastitis, progesterone, βhydroxybutyrate and urea). It has been proposed as a management tool in dairy farms (Cordes and Borchert, 2012), but the accuracy of the system to detect mastitis under field conditions is still under evaluation, and no consistent data are available.

ULTRASONIC AND ELECTROMAGNETIC SENSORS

Milk constituents can be monitored from their interactions with ultrasonic or electromagnetic waves, including either visible spectra, near-infrared spectra (NIR), and mid-infrared spectra (MIR) (Ordolff, 2005). These methods, and particularly MIR, are commonly used in laboratories to analyze for fat, protein, and lactose. MIR spectroscopy showed a high accuracy and repeatability when performed with a portable on-farm device (Svennersten-Sjaunja et al., 2005). However, due to of the limited penetration depth, which makes the spectra very sensitive to the presence of fat globules or fat biofilms, MIR spectroscopy is unsuitable for on-line raw milk analysis. NIR in comparison to MIR has a cheaper sensor, although less accurate, and because it requires no sample preparation, it is particularly suitable for on-line use (Brandt et al., 2010). A commercial on-line NIR analyzer (IMA, AfiMilk, S.A.E Afikim, Israel) that uses the wavelength region between 450 and 950 nm measures milk solid concentrations as well as the presence of blood and somatic cells in milk and is available commercially (Katz et al., 2007; Leitner et al., 2012). Consistent data are not available to assess the accuracy of the system in detecting mastitis under field conditions.

THERMOGRAPHY

Infrared thermography (IRT) is a non-invasive technique, which can be done without touching the object of measurement, thus allowing temperature detection in animals in their environment. Recent advances in thermal imaging technology have produced lightweight, portable systems that store digital images, thus increasing the potential for its use in veterinary medicine (McCafferty, 2007).

In this area, two of the most useful applications of IRT are monitoring teat tissue conditions and clinical mastitis diagnosis. Indeed, the application of IRT for the assessment of teat tissue conditions is one of the first examples of the application of IRT in veterinary medicine. Evaluation of teat tissue condition is important to investigate the interactions between the milking machine and the teat in mastitis pathogenesis studies (Hamann, 1985; Ordolff, 2000). Moreover, as previously reported, clinical mastitis leads to both systemic and local signs in the udder quarter, including an increase in skin and tissue temperatures, and radiated heat emitted by the udder can be detected with IRT (Hovinen et al., 2008). A predictive model for the temperature of the udder surface based on consecutive measurements of healthy cows and ambient temperature was developed, showing that IRT could be useful for early detection of mastitis (Berry et al., 2003).

ASSESSMENT OF TEAT CONDITION AND MILKING PROCESS

Early studies showed that skin temperature can be used to estimate tissue integrity since it reflects the underlying circulation and tissue metabolism and IRT has been adopted to study temperature patterns of udder and teat skin (Hamann, 1985, 1987; Hamann et al., 1994).

Teat integrity may be assessed either by comparing the actual temperature or relative temperature between adjacent teats or by comparing the teat circulatory response to different milking procedures (Paulrud et al., 2005). In one study, teat temperatures were measured at various times at different teat levels with different milking liners (Paulrud et al., 2005) (Table 2).

During manual udder preparation, including pre-stripping and wet cleaning, teat temperature dropped approximately 1.5°C (Paulrud et al., 2005). These changes can be explained by the removal of blood from teat veins in order to open the occlusion between udder and teat sinus and to increase the volume of the teat sinus. Due to reduced blood volume, the teat wall gets colder and the teat temperature may decrease. Temperature changes can also be explained by the fact that teat stimulation will decrease the sympathetic tone of the mammary gland resulting in increased blood flow and a reduced rate and amplitude of teat and teat sphincter muscle contraction resulting in reduced blood flow in the teat tissue (Paulrud et al., 2005). A third possibility to explain the decrease in teat skin temperature after preparation may be the activation of the autonomous nervous system and an increase in sympathetic tone, thus causing hemodynamic changes (Paulrud et al., 2005).

Table 2: Least square means of teat temperature of teat milked with two different liners. Temperatures were taken at various teat levels and in different milking phases (Paulrud et al., 2005).

Milking phase	Extended liner			Soft liner		
	Base	Mid	Tip	Base	Mid	Tip
Pre-teat preparation	35.2	34.1	33.1	35.0	33.7	32.7
Post-teat preparation	33.7	32.7	31.4	33.3	32.2	31.1
End of Milking	35.4	35.9	33.8	35.0	35.1	32.6
Milking+20 min	36.0	35.6	34.7	35.6	34.8	33.8

The absolute temperatures of the teats after milking and 20 minutes after milking were significantly higher in teats milked with the extended than with the soft liner. During milking, mid-teat temperature increased markedly, while both teat base and teat tip temperatures tended to increase less, or even slightly decrease. A decrease in tone during milking causes a marked increase of blood flow and therefore an increase of the convective heat loss from the skin (Hamann, 1985; Paulrud et al., 2005).

Temperature conditions during milking can be affected by the milk flow through the teat lumen, by the enclosure of the teat in the teatcup, and by the reactions in the cutaneous vascular plexus (Isaksson and Lind, 1994). These observations suggested that the greater the difference between pre-milking and post-milking temperatures, and the longer those differences exist, the greater impairments on teat circulation the process of milking has caused (Isaksson and Lind, 1994; Paulrud et al., 2005).

The difference in teat temperature measured pre and post milking in different conditions (Paulrud et al., 2005) may indicate that machine milking induces long lasting alterations in teat fluid dynamics and that these alterations can be assessed by IRT.

Measuring udder skin temperature by IRT should take into account the influence of several factors such as environment, cow behavior and climate. These factors could lead to day-to-day variations (Berry et al., 2003). These authors designed a study with the objective of determining the magnitude and pattern of udder temperature variation as the basis for future development of an early detection method for mastitis. Daily fluctuations in udder temperature, and the influence of environmental factors upon these values in non-mastitic cows, were assessed by IRT. Udder temperature rose significantly after an exercise period and the within-day pattern showed a distinct circadian rhythm. Lag regression analysis showed that previous daily udder temperatures together with environmental temperature parameters could successfully predict current udder temperature with a high degree of accuracy (Berry et al., 2003).

THERMOGRAPHY TO DETECT MASTITIS

There is an increasing interest in using IRT both cow-side or applied in AMS or conventional milking machines as a tool to detect mastitis. A thermal camera installed in a milking or feeding parlor might be able to detect clinical mastitis with fever before milking, thus avoiding the delivery of milk from a diseased cow to the dairy factory (Hovinen et al., 2008).

Few studies are available and their results are not consistent. The reason for these inconsistencies is the lack of standardization both in IRT measurement and, more importantly, on the definition of mastitis. As for other sensors, both these aspects must be considered in the design of new studies assessing the accuracy of IRT in detecting mastitis.

One of the first studies available (Barth, 2000) was designed to evaluate the use of IRT to detect udder inflammation. Over a period of eight days, 98 IRT images of udders from six cows were taken before morning milking and following a milking interval of 12 h. Mean surface temperature of 24 teats increased from 30.1 °C at the tip to 35.1 °C at the udder base. Significant differences of surface temperature, dependent on measurement perspective of teats (medial, lateral or caudal), were also observed. The temperature of the cistern area had the smallest variability and was used to compare the IRT temperatures of quarters. A significant temperature difference between the quarters with a SCC below and above 100,000/ml (33.6 vs. 34.1 °C) was observed, but the presence of the measurement perspective bias cannot be ignored (Berry et al., 2003). This supports the previous suggestion that IRT was unsuitable for the early detection of subclinical mastitis (Barth, 2000).

In an experimentally LPS-induced mastitis trial in 6 cows, IRT was evaluated as a tool for the early detection of the disease (Hovinen et al., 2008). Even if IRT applied to other body sites could detect febrile status, local inflammatory changes of the udder were not detected with IRT (Hovinen et al., 2008). These results were confirmed in a later study where IRT was not successful in the early detection of experimentally induced *E. coli* mastitis (Pezeshki et al., 2011).

If IRT showed to be a poor diagnostic tool to detect *E.coli* mastitis under experimental conditions, other studies in different conditions reported more promising results. In a study including 62 Brown Swiss dairy cows, IRT was compared to SCC observed at quarter level (Polat et al., 2010). The results of the study are presented in Table 3, where sensitivity and specificity values are calculated for different SCC thresholds. The study shows that a quarter with subclinical mastitis had a 2.35°C greater skin surface temperature than healthy quarters. The ROC curve calculation showed as sensitivity and specificity of IRT (95.6 and 93.6%, respectively) were not different from those for California Mastitis Test (88.9 and 98.9%, respectively).

Table 3: Sensitivity, specificity and predictive values calculated at different temperature cut-offs to detect subclinical mastitis (Polat et al., 2010).

SCC (x 1000/ml) threshold	Cut-off °C	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
>200	>34.7	83.5	100	100	73.7
>400	>34.7	95.6	93.6	95.0	93.6
>600	>35.3	89.3	92.5	91.5	90.5
>800	>35.4	90.3	89.5	85.4	96.1
>1000	>35.6	93.4	89.7	82.6	96.3
>1500	>35.8	85.4	91.5	78.8	94.4

A more recent study evaluated the factors potentially affecting IRT measurements in milking parlours and the accuracy in detecting udder diseases (Franze et al., 2012). The results showed that surface temperature measured by infrared thermography is mainly influenced by ambient temperature and time of day, being higher at the evening milking than at the morning milking. Other factors whose effects were considered included air pressure, relative humidity, wind speed and cleanliness.

Cows with udder disease show significantly different infrared temperatures of rear udder quarters than cows with healthy udders. Cows with a latent infection, unspecific mastitis and mastitis of rear udder quarters can be detected by daily infrared thermography with a sensitivity of around 30% and a specificity of around 70%, if the change of surface temperature is considered individually for the animal.

CONCLUSIONS

The changes in herd size, management and efficiency increase the needs for new technologies to help farmers to identify problems as early as possible in a simple and efficient way. In this area, a lot of effort has been devoted to develop sensors able to identify diseases such as mastitis. Indeed, mastitis is still one of the most important and costly diseases in dairy herds and its control is based primarily on its accurate and early detection.

There is not a consensus of opinion on the performance criteria that should be fulfilled by the sensor system to be applied to detect mastitis. Moreover, little attention has been paid to what farmers need as reported by a recent paper (Mollenhorst et al., 2012). This paper clearly showed that farmers would prefer a detection system that produces a low number of false alerts and gives alerts for the more severe cases which are not too late for remedial action to be taken (Mollenhorst et al., 2012). One of the most important criteria for a tool to detect clinical mastitis is to identify a potential problem cow early enough to be able to cure the diseased cow and in this case a slightly higher false alert rate would not be a problem (Hogeveen et al., 2010).

IRT would fit very well within this framework, being simple, non-invasive and cheap to perform. It has already been shown to be a useful tool to evaluate tissue changes induced by milking machines. However, when mastitis detection is considered, the available results are not consistent and a sufficient level of accuracy has not been demonstrated. These negative results are not due only to a poor performance of methodologies based on IRT, but also to a poor definition of the key parameters and characteristics. Indeed, as for other sensors applied for mastitis detection (Mollenhorst et al., 2012), no consensus exists about the real gold standard, and almost every study has used a different time window and site of assessment at udder level.

IRT has a vast potential to become a tool to diagnose udder and tissue inflammation in response to mechanical and microbial stressors. However, to achieve this goal, better designed, more accurate studies are needed. The possibility of using IRT both cow-side and in automatic milking systems as a diagnostic tool not only for mastitis detection, but also in other areas of dairy herd management supports the interest in developing its applications. These aspects coupled with the fast-growing improvements in this technology make IRT one of the most promising techniques for on-line management of modern dairy herds.

REFERENCES

1) Alsaaod, M. and W. Buscher. 2012. Detection of hoof lesions using digital infrared thermography in dairy cows. Journal of Dairy Science 95(2):735-742.
2) Barth, K. 2000. Basic investigations to evaluate a highly sensitive infrared-thermograph-technique to detect udder inflammation in cows. Milchwiss.-Milk Sci. Int. 55 (11) : 607-609.
3) Berry, R. J., A. D. Kennedy, S. L. Scott, B. L. Kyle, and A. L. Schaefer. 2003. Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis detection. Canadian Journal of Animal Science 83(4):687-693.
4) Brandt, M., A. Haeussermann, and E. Hartung. 2010. Invited review: Technical solutions for analysis of milk constituents and abnormal milk. Journal of Dairy Science 93(2):427-436.

5) Chagunda, M. G. G., N. C. Friggens, M. D. Rasmussen, and T. Larsen. 2006. A model for detection of individual cow mastitis based on an indicator measured in milk. Journal of Dairy Science 89(8):2980-2998.
6) Cordes, D. and U. Borchert. 2012. Modern trends of milking systems to support udder health - case study Herd Navigator (TM). Arch. Lebensm.hyg. 63(3):65-67.
7) Franze, U., S. Geidel, U. Heyde, A. Schroth, T. Wirthgen, and S. Zipser. 2012. Investigation of infrared thermography for automatic health monitoring in dairy cows. Zuchtungskunde 84(2):158-170.
8) Friggens, N. C., M. G. G. Chagunda, M. Bjerring, C. Ridder, S. Hojsgaard, and T. Larsen. 2007. Estimating degree of mastitis from time-series measurements in milk: A test of a model based on lactate dehydrogenase measurements. Journal of Dairy Science 90(12):5415-5427.
9) Hamann, J. 1985. Measurement of machine milking induced teat tissue reactions. Milchwiss.-Milk Sci. Int. 40(1):16-18.
10) Hamann, J. 1987. Effect of machine milking on teat end condition - a literature review. Bulletin of the International Dairy Federation 215:33-49.
11) Hamann, J., C. Burvenich, A. J. Bramley, O. Osteras, M. Woolford, M. Woyke, W. Haider, M. Mayntz, and J. Ledu. 1994. Teat tissue reactions to machine milking and new infection risk. Bulletin of the International Dairy Federation 297:1-43.
12) Hamann, J. and A. Zecconi. 1998. Evaluation of the electrical conductivity of milk as a mastitis indicator. Fil-Idf (Federation Internationale de Laiterie International Dairy Federation) Bulletin. 1998. No. 334, 5-22. 57 ref.
13) Hogeveen, H., C. Kamphuis, W. Steeneveld, and H. Mollenhorst. 2010. Sensors and Clinical Mastitis-The Quest for the Perfect Alert. Sensors 10(9):7991-8009.
14) Hogeveen, H. and W. Ouweltjes. 2003. Sensors and management support in high-technology milking. J. Anim. Sci. 81:1-10.
15) Hovinen, M., J. Siivonen, S. Taponen, L. Hanninen, M. Pastell, A. M. Aisla, and S. Pyorala. 2008. Detection of Clinical Mastitis with the Help of a Thermal Camera. Journal of Dairy Science 91(12):4592-4598.
16) I.D.F. 1995. Milk: Enumeration of Somatic Cells. International IDF Standard 148A:1-8.
17) Isaksson, A. and O. Lind. 1994. Milking related changes in the surface temperature of the bovine teat skin. Acta Veterinaria Scandinavica 35(4):435-438.
18) Katz, G., A. Arazi, N. Pinsky, I. Halachmi, Z. Schmilovitz, E. Aizinbud, and E. Maltz. 2007. Current and near term technologies for automated recording of animal data for precision dairy farming. J. Anim. Sci. 85:377-377.
19) Leitner, G., U. Merin, L. Lemberskiy-Kuzin, D. Bezman, and G. Katz. 2012. Real-time visual/near-infrared analysis of milk-clotting parameters for industrial applications. Animal 6(7):1170-1177.
20) McCafferty, D. J. 2007. The value of infrared thermography for research on mammals: previous applications and future directions. Mammal Rev. 37(3):207-223.
21) Mollenhorst, H., L. J. Rijkaart, and H. Hogeveen. 2012. Mastitis alert preferences of farmers milking with automatic milking systems. Journal of Dairy Science 95(5):2523-2530.
22) N.M.C. 1996. Current Concepts of Bovine Mastitis. N.M.C., Madison WI USA.
23) Ordolff, D. 2000. Surface temperatures of udder and teats for conventional and automatic milking methods. Kieler Milchwirtsch. Forschber. 52(1):5-10.
24) Ordolff, D. W. 2005. On-farm-analysis of milk: a promising challenge. Precision Livestock Farming '05. Wageningen Academic Publishers, Wageningen.
25) Paulrud, C. O., S. Clausen, P. E. Andersen, and M. D. Rasmussen. 2005. Infrared thermography and ultrasonography to indirectly monitor the influence of liner type and overmilking on teat tissue recovery. Acta Veterinaria Scandinavica 46(3):137-147.

26) Pezeshki, A., P. Stordeur, H. Wallemacq, F. Schynts, M. Stevens, P. Boutet, L. J. Peelman, B. De Spiegeleer, L. Duchateau, F. Bureau, and C. Burvenich. 2011. Variation of inflammatory dynamics and mediators in primiparous cows after intramammary challenge with Escherichia coli. Veterinary Research 42.
27) Polat, B., A. Colak, M. Cengiz, L. E. Yanmaz, H. Oral, A. Bastan, S. Kaya, and A. Hayirli. 2010. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. Journal of Dairy Science 93(8):3525-3532.
28) Rainwater-Lovett, K., J. M. Pacheco, C. Packer, and L. L. Rodriguez. 2009. Detection of foot-and-mouth disease virus infected cattle using infrared thermography. Vet. J. 180(3):317-324.
29) Schaefer, A. L., N. J. Cook, C. Bench, J. B. Chabot, J. Colyn, T. Liu, E. K. Okine, M. Stewart, and J. R. Webster. 2012. The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. Res. Vet. Sci. 93(2):928-935.
30) Stokes, J. E., K. A. Leach, D. C. J. Main, and H. R. Whay. 2012. An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foot lesions in dairy cattle. Vet. J. 193(3):674-678.
31) Svennersten-Sjaunja, K., M. Sjogren, I. Andersson, and L. O. Sjaunja. 2005. Milk analyses: a comparison between a simple IR-instrument for use on farm level and available IR-methods. Precision Livestock Farming '05. Wageningen Academic Publishers, Wageningen.
32) Zecconi, A., M. Di Pietro, A. Fantini, and F. Sangiorgi. 2010. Mastite bovina. Manuale pratico per un efficace controllo in stalla. Edizioni L'Informatore Agrario, Verona.

INFRARED THERMOGRAPHY IN REPRODUCTION

CALOGERO STELLETTA, JURI VENCATO, ENRICO FIORE, MATTEO GIANESELLA

Department Animal Medicine, Production and Health, Università degli Studi di Padova, Italy

ABSTRACT

Thermography has been applied many times for studies carried out on “human” and “animal” reproduction. Numerous of these had as objective the identification of some specific pathological conditions through the use of a thermographic monitoring protocol.

Thermographic approach to the reproductive pathological condition influencing the body or local cutaneous areas can be routinely applied in veterinary medicine only after a specie-specific definition of the emission index of each cutaneous area interesting for diagnostic procedures. Some scientific works reported the possibility to identify cows during the estrous period or the pregnancy status. During the last decade the Authors had been performing different experiments to set up these specie-specific characteristics. At the start time, the objective was to create a strong relation among different imaging diagnostic tools usually utilized in veterinary medicine considering the also the thermographic approach. Among the studies carried out by our group, some reported specific thermographic monitoring sessions for the optimization of hormonal treatments during ovarian synchronization protocols in the mare and the ewe. In general, cutaneous temperature of vulvar and peri-vulvar areas may be useful for increase the awareness about the ovulation time. In these areas, the thermal radiation is related to the cutaneous or sub-cutaneous blood flux variations. During the estrous time an increase of blood efflux is usually depending by the level of secreted estrogens by the growing ovarian follicles. Because of its no-invasivity and the high sensitivity in measure temperature differences across the skin surface it appears clear that thermography could be applied to the monitoring of scrotal surface temperature. An extensive literature about thermography application in diagnosis, and follow-up monitoring in case of varicocele is available today. As a consequence of the use in human andrology, there was an increasing interest of animal andrologist on infrared thermography and the first studies had came out in the eighties. They showed like thermography could be used to assess scrotal/testicular thermoregulation in bull by providing an image on the base of the infrared emissions with an accuracy of 0.10°C.

Key words

Thermography, reproduction, veterinary medicine, cutaneous or sub-cutaneous blood flux variations, thermoregulation.

Thermography has been applied many times for studies carried out on human and animal reproduction. Many of these studies had, as the main objective, the identification of some specific pathological conditions through the use of a thermographic monitoring protocol. Some studies, published for human medicine, described the use of this method for the early diagnosis of cases of uterine cervix lesions in women (Smaga et al. 2003). It has also been reported that thermal cameras have been used to increase research and application on reproduction in carnivores (Durrant et al., 2006). Unfortunately, at the present time, nobody uses this technique as principal diagnostic tool for reproductive diseases, where it is usually considered for supplementary or ancillary examination to increase the diagnostic precision. The main constraint to the common use of the thermographic monitoring for diagnostic purposes is the absence of specific protocols for its application. A number of image analysis software packages are available and can be modified depending on the needs of the operator. The operator has to understand fully how to operate the thermal camera and what type of measurements have to be considered in each environmental condition. Unfortunately, the potential measurement errors related to the individual animal variations block the set up of species-specific software. Individual variations are usually linked to the presence of some factor influencing the infrared emission from a cutaneous area (species-specific factors like hair length, cutaneous vascular bed amplitude and thickness) while the environmental factors can be considered by the thermal camera settings protocol. Continuing research activities on the definitive interspecies differences in terms of thermal characteristics (cutaneous thermal exchange equation) may help in resolving these problems in practical and diagnostic applications.

A thermographic approach to a reproductive pathological condition influencing the body or local cutaneous areas can be routinely applied in veterinary medicine only after a species-specific definition of the emission index of each cutaneous area of interest for diagnostic procedures. Some scientific research has reported the possibility of identifying cows during the oestrous period or their pregnancy status.

Hurnik et al. (1985) studied the relationship between differences in body surface temperatures and oestrus in Holstein-Friesian dairy cows, and the possibility of using this technique to determine the onset of oestrous. Because inaccuracies were encountered in determining the oestrous cycle during the experiment, the authors did not recommend thermographic for routine detection of oestrous, but it is nevertheless completely adequate in skin temperature studies, or, more precisely, in the studies of body surface temperature changes. Hellebrand et al (2003) concluded that the stage of pregnancy of heifers in their usual environment (pasture or barn) cannot be determined by simple monitoring with a thermal imager, but they did find that the external temperature of the genitalia follows the core body temperature, and thus thermography can be utilized for oestrous climax determination.

During the last decade the authors have been performing different experiments to set up these species-specific characteristics. Initially the objective was to create a strong relation between different imaging diagnostic tools often used in veterinary medicine considering also the thermographic approach. Among the studies carried out by our group, some reported specific thermographic monitoring sessions for the optimization of hormonal treatments during ovarian synchronization protocols in the mare and the ewe. In general, cutaneous temperature of vulvar and peri-vulvar areas may be useful for increasing the awareness of the ovulation time. In these areas, thermal radiation is related to the cutaneous or sub-cutaneous blood flux variations. During oestrous an increase of blood efflux is usually dependent upon the level of oestrogens secreted by the growing ovarian follicles.

In a recent study performed by our group (unpublished data) a monitoring protocol was designed (i) to determine the effect of two GnRH analogues (Ovsynch protocols) administered at two different times of a day for oestrous synchronization, and (ii) to study the relation of vaginal electrical impedance and vulvar and perivulvar temperature recorded with infrared thermography with pregnancy rates in Italian Mediterranean buffaloes inseminated with sexed frozen semen. Thirty-two healthy Mediterranean buffaloes with variable post-partum period (16 to 174 day) were used. Before starting the synchronization protocol, a trans-rectal ultrasound examination had been carried out to identify the follicular status in the ovaries. Follicles ≥3mm in diameter and the corpus luteum(CL) were measured and recorded. The Ovsynch protocol (Pursley et al., 1995; De Rensis & Lopez Gatius, 2007) was started regardless of the stage of the oestrus cycle in animals. All the female buffaloes were inseminated with sexed frozen semen (X-chromosome bearing spermatozoa), from a bull of known high fertility, 18-21 hours from administration of the second dose of GnRH (Figure 1.). There was a well defined temperature drop between the 2nd administration of GnRH and the time of insemination only in buffaloes pregnant at 42 days after artificial insemination (AI). This type of thermal profile during an Ovsynch protocol may be easily explained. The 2nd administration of GnRH represents a hormonal input that simulates the natural pre-ovulatory surge and, 18-21 hours after, only the conceiving females were at the perfect time for AI. The decrease in vulvar temperature during that period may be related to a natural decrease of the circulating oestrogens in the immediately preovulatory period creating a good environment for the sperm oviductal reservoir and the subsequent fertilization.

Essential pre-requisites for successful artificial insemination are the accurate detection of oestrous and ovulation, which is classically performed using a "teaser", trans-rectal palpation and ultrasonographic examination of reproductive tract. An alternative method could be the measurement of electrical impedance of vaginal mucus and perivulvar and vulvar temperature. Reports on using infrared thermography in detection of oestrous in the mare are limited, so the aim of this study was to assess perivulvar and vulvar temperature using infrared thermography as a non-invasive method for the monitoring of the oestrous cycle in the mare. Nine trotter mares were used, five with foal and four without foal. The thermographic monitoring was considering a three stages protocol: T1 (follicle with 0 > 3cm diameter), T2 (follicular growth), T3 (ovulation). At each scanning, the thermography was performed first on perivulvar and vulvar regions by Thermacam P25, followed by trans-rectal palpation and ultrasonographic examination of the reproductive tract using an 8Mhz ultrasound probe. Finally, the measurement of electrical impedance was performed using an endo-vaginal Draminisky probe. Ten blood samples were collected from five mares to measure serum progesterone and oestrogen concentration in stages T2 (5) and T3 (5).

The analysis showed a positive correlation within the thermographic parameters, which were Perivulvar Maximum Temperature (PMaxT), Perivulvar Mean Temperature (PMeanT), Vulvar Maximum Temperature (VMaxT), Vulvar Mean Temperature (VMeanT). There was a simultaneous increase of these parameters (Table 1.). These parameters that increased were positively correlated with the Diameter of the Greater Follicle (DGF) and the Echotexture of the Follicular Wall (EFW), and negatively correlated with the presence of corpora lutea (CL). These data suggest an increase of maximum and mean perivulvular and vulvar temperature during follicular growth and a decrease in temperature during the establishment of CL. These changes may be because the mare, under the influence of oestrogen, has an increase of hyperemia of the vulvar region. A negative correlation was also found between PMT and the values of Electrical Impedance (EI). The increase of PMT is associated with lower values of EI, which occur during the follicular growth. Moreover, the PMT was positively correlated with serum oestrogen concentration and negatively correlated with serum progesterone concentration which occurs during the follicular growth and ovulation times respectively. The authors (Calabria et al. 2010) concluded that their preliminary results are encouraging the possible use of thermography as an auxiliary noninvasive method during oestrous cycle monitoring in mares.

In other work (Stelletta et al., 2006) reported the use of thermographic monitoring in ewes during a classical hormonal treatment to synchronize the ovulation. The aim of that study was to detect skin temperature differences of the perivulvar area between ewes in oestral and anoestral phases. Twenty-four dairy ewes, 16 in oestral phase and 8 in anoestral phase, were investigated. Oestrus was synchronized using intravaginal progestagen-impregnated sponges (fluorogestone acetate, FGA) for 14 days and after the sponges were removed, ewes were treated with PMSG (pregnant mares serum gonadotrophin). Thermography sessions were carried out 50 hours after sponges were removed, from a distance of 1 m from the vulvar area. A skin emissivity of 0.95 was assumed. The control subjects with no synchronization treatment were in a seasonal anoestral period. The analyses performed on the resultant thermograms were (i) a qualitative and quantitative analysis taking into account the mean perivulvar area temperature, (ii) a quantitive analysis of the temperature differences between unfleeced adjoining areas, and (iii) a quantitative frequency analysis of temperature using an interval of 0.2°C. A significant difference between the two groups was observed in values expressed as mean temperature of perivulvar area ($P < 0.05$). The subjects in oestrous and in anoestrus ranged in temperature from 35.9°C to 37.7°C with an average of 36.9 ± 0.5°C and 34.2°C to 36.5°C with an average of 35.42 ± 0.63°C respectively. The superficial temperatures detected in unfleeced areas of posterior anatomical regions may be taken into account in the study of circulatory and/or hormonal variations in ewes. The mammary skin receives only 2% of the regional blood flow and so this area may be used as a control for other adjoining areas because the emitted heat increase is proportional to the parenchymal blood flow. The animals in oestrous show an increase in blood flow to the genitals and hormonal changes. These variations are able to explain the increase of heat emitted by the unfleeced skin of posterior areas. Thermography is a technique useful to point out the different nature of heat transmission from underlying areas of the skin. The ewes with oestrous induced by synchronization have shown a different thermal behavior that can be detected by thermography sessions in adjoining areas receiving blood from common arteries (internal iliac –pudendal artery) (Figure 2.).

Infrared thermography application in veterinary andrology

The first applications of infrared thermography in human andrology date back to the 1970s. Scrotal thermography has been demonstrated, since that time, to be a useful diagnostic method, especially in varicocele (Comhaire et al., 1976; Comhaire, 1977) and testicular tumors (Lee and Gold, 1976). In particular, the relation between temperature elevation and seminal quality was known more than twenty years before (Hanley, 1956). Because it is non-invasive and has high sensitivity in measuring temperature differences across the skin surface, it appears clear that thermography could be applied to the monitoring of scrotal surface temperature (Kulis et al., 2012). Extensive literature is available about the application of thermography in the diagnosis and follow-up monitoring in cases of varicocele. Because of its use in human andrology, there has been an increasing interest from animal andrologists in using infrared thermography and the first studies were reported in the 1980s. Research showed that thermography could be used to assess scrotal/testicular thermoregulation in bull by providing an image based on the infrared emissions with an accuracy of 0.10°C (Coulter, 1988; Purohit et al., 1985). Researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature (Coulter et al., 1988) and that infrared temperature thermograms provide accurate information about testicular thermoregulation (Coulter, 1988; Kastelic et al., 2001; Purohit et al 1985; Lunstra and Coulter, 1997).

In contrast with human colleagues, veterinarians are faced with more difficult conditions because of the “patients” and the working environment. Kastelic et al. (1996d) studied the effect of environmental factors affecting the measurement of bovine scrotal surface temperature with infrared thermography. Although it is established that many biological systems have diurnal rhythms, no significant changes were found on scrotal surface temperature, so scrotal thermography can be performed at any time of the day. Feeding had been shown to alter thermography. Within 30 minutes after the start of feeding an increase of scrotal temperature can be observed, with an effect lasting for several hours. Recumbency can have an effect depending on ambient temperature and floor material. In conditions of high environmental temperatures, cool floors, such as rubber mats and metal gratings, may absorb heat from the scrotum, resulting in scrotal warming after rising. Ambient temperature has a great effect on the surface temperature of the lower surface of the scrotum, a small effect on the top of the scrotum and an intermediate effect on the average scrotal temperature. In particular, a rapid change in ambient temperature leads to an apparent overcompensation response 3 hours later, followed by a slow return to normal temperature after 23 hours. Moisture on the scrotum decreases scrotal temperature and at least 30 minutes following drying is required for the temperature to return to normal. Once the limitations are fully understood the thermography has been demonstrated to be a sufficiently robust technique (Kastelic et al., 1996d).

In normal conditions, scrotal surface temperature of a bull is 5-6°C lower than abdominal temperature (Arteaga et al., 2005; Brito et al., 2004; Brito et al., 2003; Kastelic et al., 1996a; Kastelic et al., 1997) and has a positive top-to-bottom gradient, with the top warmer than the bottom (Figure 3)(Kastelic et al., 1996a). This gradient is due to the scrotum being vascularized from the top to the bottom. Conversely, the testicular artery, once it reaches the ventral pole of the testis, diverges into several smaller arteries before entering the testicular parenchyma (Setchell, 1970). There should thus be a negative temperature gradient but the opposing temperature gradient of the scrotum complements that within the testis, resulting in a relatively uniform intratesticular temperature (Kastelic et al., 1995; Kastelic et al., 1996a). Scrotal temperature of the area overlying the cauda epididymis increased following ejaculation and electroejaculation due to the contraction of the cauda epididymis during ejaculation (Kastelic et al., 1996b). Most reported studies have been conducted in bulls, working in two main areas: the study of thermoregulation and its relation to testicular activity and the use of thermography in monitoring scrotal surface temperature to predict fertility.To record a good scrotal thermogram it is necessary to hold the infrared scanner approximately 1 m behind the bull and to orient the scanner perpendicularly to the paired testes in the scrotum (Brito et al., 2012; Lunstra and Coulter, 1997).

Environmental factors affecting bull thermoregulation

Techniques for studying the effects of heat on the testis include a whole body heating or a local heating of the testis. The first technique consists of exposing the whole animal to a hot environment (Setchell, 1998), however there are two complicating factors with this approach. Firstly, the body reacts to heat stress in a variety of ways that involves physiological, metabolic and endocrinological changes that can affect indirectly the testis. Secondly, the sweat glands of the scrotum are not controlled independently of those of the general body surface (Robertshaw and Vercoe, 1980), so the ability to produce sweat can be influenced by the prior heat exposure. Kastelic et al. (1997) used this technique to study testicular thermoregulation in two different ambient temperatures. Scrotal surface temperature was shown to be influenced by external temperature with an increasing of about 2.5°C when passing from 15°C to 25°C either in the top, bottom or average temperature. The temperature gradient was not affected. The same experiment was than performed after castration resulting in the evidence that the testis has a limited influence on scrotal surface temperature (Kastelic et al., 1997).

Local heating of the testes has usually been achieved in one of three ways, induced cryptorchidism, scrotal insulation or short-term heating, usually by immersion in a water bath (Setchell, 1998). Of these techniques, the most useful in thermography studies is scrotal neck insulation (Brito et al., 2003; Kastelic et al., 1996c). Kastelic et al. (1996c) used an infrared thermal camera to assess the insulation effect on scrotal surface temperature. Images were taken prior to insulation and then at 24 and 48 hours after insulation. At the same time they monitored scrotal subcutaneous temperature, intratesticular temperature and semen quality. Insulation resulted in a decreased scrotal surface temperature of the top of the scrotum after 24 hours, similar to the neck, and an increased temperature of the bottom scrotal area. After 48 hours, temperature returned to the same level as pre-insulating, suggesting some compensatory mechanism. Despite the return to a normal scrotal temperature an increased intratesticular temperature was observed at 48 hours, presumably because heat radiation from the testicular vascular cone and possibly countercurrent heat exchange were impaired (Kastelic et al., 1996c). Insulation was also correlated with a decreased of seminal parameters, particularly an increase in abnormal sperm (Barth and Bowman, 1994; Brito et al., 2003; Fernandes et al., 2008; Kastelic et al., 1996c). Insulation of the neck of the scrotum may be also a suitable model for simulating over conditioned bulls with a large amount of fat in the scrotal neck (Coulter and Kastelic, 1994). Dietary energy is another factor that affects scrotal thermoregulation and seminal quality (Coulter et al., 1987). Thermography was used to assess the dietary energy's effect on male reproduction (Coulter et al., 1997). After a period of 168 days after weaning, scrotal surface temperature was measured in different lines of beef bulls fed with moderate or high-energy diets. The authors did not find a significant effect of diet on the top, bottom or average scrotal surface temperature, but there was an effect on the gradient, with the bulls on the high-energy diet having a smaller gradient than those on the moderate-energy diet (3.4 vs 3.9°C) (Coulter et al., 1997).

Bull Breeding Soundness Evaluation

Starting from the middle of 1990s, infrared thermography had been proposed as a support tool in the evaluation of breeding soundness in bulls. In some studies scrotal surface temperature was evaluated in basal conditions (Kastelic et al., 2001; Lunstra and Coulter, 1997) and in others its use was related to the testicular response to an exogenous GnRH administration (Gabor et al., 1998a; Gabor et al., 1998b; Vencato et al., 2012). In the first case, thermograms were taken prior to semen collection and then data were correlated with other factors including seminal parameters, testicular echotexture, testicular tone and scrotal circumference. Lunstra and Coulter (1997) described three possible classes on the base of scrotal thermogram. Numerous horizontal bands, with each band representing a narrow temperature range and reflecting progressively cooler temperatures as distance away from the body increased, characterize normal pattern. Scrotal thermograms that displayed some non-uniformity of band width or some asymmetry of bands can be classified as questionable. Scrotal thermograms that displayed very few bands, more marked disruption of band uniformity, or pronounced band asymmetry (hot spots) classified as abnormal (Lunstra and Coulter, 1997). Bulls exhibiting abnormal scrotal temperature patterns had a lower percentage of sperm with normal head morphology, tail morphology, and acrosome morphology, and had a higher percentage of sperm with proximal droplets when compared with bulls with normal or questionable thermograms patterns (Lunstra and Coulter, 1997). The authors concluded that bulls with abnormal scrotal temperature patterns achieved significantly lower pregnancy rates when use for natural mating, and that infrared thermography can be used to predict reduced fertility (Lunstra and Coulter, 1997).

The purpose of using thermography during a GnRH stimulation test is to determine the change in scrotal surface temperature and to relate this change to male fertility (Gabor et al., 1998a; Gabor et al., 1998b; Vencato et al., 2012). In those studies a thermogram was taken immediately before the GnRH administration and after 45 minutes (Gabor et al., 1998a; Gabor et al., 1998b) or every 15 minutes until 1 hour after GnRH administration (Vencato et al., 2012) (Figure 4). Gabor et al. (Which reference year), working with sexual mature bulls, found that 45 minutes after GnRH administration there was an increase in scrotal surface temperature. They also described an increasing in LH and Testosterone concentration after GnRH administration. There were also significant correlations between the number of spermatozoa and the percentage of live spermatozoa and other end points including thermographic measures. The authors concluded that infrared thermography is useful for predicting the number and percentage of live spermatozoa (in association with testicular size and echotexture) (Gabor et al., 1998a). Vencato et al. (2012) performed GnRH testing in young bulls with poor semen production. In their study bulls could be divided in two groups in relation to the variations of scrotal surface temperature at 60 minutes after GnRH administration. A group that had a significant decrease of scrotal temperature and a group that had a significant increase in temperature compared to scrotal surface temperature before GnRH administration. Although in both groups there was an increase in serum testosterone level, the amount of the increment was significantly higher in the group with a decreased scrotal temperature after GnRH administration. In the weeks after GnRH administration there was an increase in quality parameters of semen, and again the increment was higher in the group with the decreased temperature after GnRH administration (Graphic 1). Analysis of Pearson correlation indexes revealed a significant negative correlation between testosteronemia and scrotal surface temperature variation pre- to post-GnRH administration (Vencato et al., 2012).

Other species

There are few reported studies from other species.

Thermography was recently used (Ramires-Neto et al., 2012) to study, in stallions, the efficiency in testicular thermoregulation. Stallions of different ages were conditioned in an environment with an ambient temperature of 30-32°C. The results of the research demonstrate that normal stallions of different ages can maintain a constant testicular temperature even under heat stress conditions, since no difference in scrotal surface temperature was detected. The authors concluded that thermography has potential for use as a complementary examination technique in andrological evaluations.

Thermography was used to monitor scrotal surface temperature changes in response to GnRH administration in male alpacas (Stelletta et al., 2009). The authors performed GnRH on male animals in different conditions: after female isolation (T1), after female exposure (T2) and after female exposure with mounting (T3). The measured scrotal temperatures are reported (Graph 2) and shown that in all groups a decreased temperature was observed after GnRH administration. Moreover, a significant negative correlation was found between scrotal temperature variation and testosterone variation after GnRH administration suggesting that thermography can be use to assess the effect of GnRH administration (Stelletta et al., 2012).

Thermography had also been used to evaluate the safety of testicular biopsies in llamas (Heath et al., 2002). Thermographic images were obtained prior to biopsy, immediately after the biopsy and then once a week for 6 weeks. The biopsy process had no effect on scrotal temperature measured with thermography, although 3 animals of 9 showed a transient alteration in the thermogram patterns 1 week after biopsy (Heath et al., 2002).

REFERENCES

1) Arteaga, A.A., A.D. Barth, and L.F. Brito. 2005. Relationship between semen quality and pixel-intensity of testicular ultrasonograms after scrotal insulation in beef bulls. *Theriogenology*. 64:408-415.
2) Barth, A.D., and P.A. Bowman. 1994. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *The Canadian veterinary journal. La revue veterinaire canadienne*. 34:93-102.
3) Brito, L.F., A.D. Barth, R.E. Wilde, and J.P. Kastelic. 2012. Testicular vascular cone development and its association with scrotal temperature, semen quality, and sperm production in beef bulls. *Animal reproduction science*. 134:135-140.
4) Brito, L.F.C., A.E.D.F. Silva, R.T. Barbosa, and J.P. Kastelic. 2004. Testicular

thermoregulation in Bos indicus, crossbred and Bos taurus bulls: relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. *Theriogenology*. 61:511-528.

5) Brito, L.F.C., A.E.D.F. Silva, R.T. Barbosa, M.M. Unanian, and J.P. Kastelic. 2003. Effects of scrotal insulation on sperm production, semen quality, and testicular echotexture in Bos indicus and Bos indicus × Bos taurus bulls. *Animal reproduction science*. 79:1-15.
6) Calabria A, Corcillo G, Valentini S, Stelletta C. 2010. Utilizzo della termografia durante la determinazione dell'estro e del momento di ovulazione nella cavalla. In: Atti VIII Congresso Nazionale S.I.R.A. Ozzano dell'Emilia, 17-18 Giugno 2010.
7) Comhaire F. 1977. Scrotal thermography in patients with varicocele. Contracept Fertil Sex 5 (7): 561-565.
8) Comhaire F, R, Monteyne, and M. Kunnen. 1976. The value of scrotal thermography as compared with selective retrograde venography of the internal spermatic vein for the diagnosis of "subclinical" varicocele. Fert. Steril. 27 (6): 694-698.
9) Coulter G.H. 1988. Thermography of bull testes. Proc 12th Technical Conference on artificial insemination and reproduction, Milwaukee, WI, 22-23 April, National Association of Animal Breeders, Columbia, MO, pp. 58-62.
10) Coulter G.H. T.D. Carruthers, R.P. Amann, and G.C. Kozub. 1987. Testicular development, daily sperm production and epididymal sperm reserves in 15-month-old Angus and Hereford bulls: Effects of bull strain plus dietary energy. *J Anim Sci*, 64:254-260.
11) Coulter G.H., and J.P. Kastelic. 1994. Testicular thermoregulation in bulls. Proc. 15th Tech. Conf. Artif. Insem. Reprod., National Association of Animal Breeders pp 28-34.
12) Coulter, G.H., R.B. Cook, and J.P. Kastelic. 1997. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. *J Anim Sci*. 75:1048-1052.
13) De-Rensis, F., F. Lopez-Gatius, 2007. Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis). A review. *Theriogenology*. 67, 209-216.
14) Durrant, B.S., N. Ravida, T. Spady, A. Cheng. 2006. New technologies for the study of carnivore reproduction. *Theriogenology.* 66(7-7): 1729-36.
15) Fernandes, C.E., M.A. Dode, D. Pereira, and A.E. Silva. 2008. Effects of scrotal insulation in Nellore bulls (Bos taurus indicus) on seminal quality and its relationship with in vitro fertilizing ability. *Theriogenology*. 70:1560-1568.
16) Gabor, G., R.G. Sasser, J.P. Kastelic, G.H. Coulter, D.O. Everson, G. Falkay, M. Mézes, S. Bozò, R.B. Cook, J. Volgyi-Csik, I. Barany, and F. Szasz Jr. 1998a. Endocrine and thermal responses to GnRH treatment and prediction of sperm output and viability in Holstein-Friesian breeding bulls. *Theriogenology*. 50:177-183.
17) Gabor, G., R.G. Sasser, J.P. Kastelic, G.H. Coulter, G. Falkay, M. Mézes, S. Bozò, J. Volgyi-Csik, I. Barany, and F. Szasz Jr. 1998b. Morphologic, endocrine and thermographic measurements of testicles in comparison with semen characteristics in mature Holstein-Friesian breeding bulls. *Animal reproduction science*. 51:215-224.
18) Hanley H.C. 1956 Surgical correction of errors in testicular temperature regulation. 2nd World Congress of fertility and sterility. Naples, p.953.
19) Heath, A.M., D.G. Pugh, E.A. Sartin, B. Navarre, and R.C. Purohit 2002. Evaluation of the safety and efficacy of testicular biopsies in llamas. Theriogenology 58:1125-1130.
20) Hellebrand, H.J., Brehme, U., Beuche, H., Stollberg, U., Jacobs, H., 2003. Application of thermal imaging for cattle management. In: Proc., 1st European Conference on Precision Livestock Farming, Berlin, Germany: 761- 763.
21) Hurnik, J.F., Webster, A.B., De Boer, S., 1985. An investigation of skin temperature differentials in relation to estrus in dairy cattle using a thermal infrared scanning technique. *J.Anim.Sci.,* 61: 1095 -1102.

22) Kastelic, J.P., R.B. Cook, and G.H. Coulter. 1996a. Contribution of scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. *J Reprod Fert*. 108:81-85.
23) Kastelic, J.P., R.B. Cook, and G.H. Coulter. 1997. Contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. *Animal reproduction science*. 45:255-261.
24) Kastelic, J.P., R.B. Cook, G.H. Coulter, and R.G. Saacke. 1996b. Ejaculation increases scrotal surface temperature in bulls with intact epididymes. *Theriogenology*. 46:889-892.
25) Kastelic, J.P., R.B. Cook, G.H. Coulter, and R.G. Saacke. 1996c. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology*. 45:935-942.
26) Kastelic, J.P., R.B. Cook, G.H. Coulter, G.L. Wallins, and T. Entz. 1996d. Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. *Animal reproduction science*. 41:153-159.
27) Kastelic, J.P., R.B. Cook, R.A. Pierson, and G.H. Coulter. 2001. Relationships among scrotal and testicular characteristics, sperm production, and seminal quality in 129 beef bulls. *Can J Vet Res*. 65:111-115.
28) Kastelic, J.P., G.H. Coulter, and R.B. Cook. 1995. Scrotal surface, subcutaneous, intretesticular, and intraepididymal temperatures in bulls. *Theriogenology*. 44:147-152.
29) Kulis, T., D. Kolaric, K. Karlovic, M. Knezevic, S. Antonini, and Z. Kastelan. 2012. Scrotal infrared digital thermography in assessment of varicocele – pilot study to assess diagnostic criteria. Andrologia 44, 780-785.
30) Lee Y.T., and R.H. Gold 1976. Localization of occult testicular tumor with scrotal thermography. JAMA 236(17): 1975-1976.
31) Lunstra, D.D., and G.H. Coulter. 1997. Relationship between scrotal infrared temperature patterns and natural mating fertility in beef bulls. *J Anim Sci*. 75:767-774.
32) Purohit, R.C., R.S. Hudson, M.G. Ridell, R.L. Carson, D.F. Wolfe, and D.F. Walker. 1985. Thermography of the bovine scrotum. Am J Vet Res 46:2388-2392.
33) Pursley, J.R., M.O. Mee, M.C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. *Theriogenology*. 44, 915-923.
34) Ramires-Neto, C., G.A. Monteiro, D.J.Z. Delfiol, E.G: Fioratti, J.A. Dell'Acqua Jr., F.O. Papa, and M.A. Alvarenga. 2012. Study of testicular thermoregulation efficiency in stallion with different ages. 8th ISSR Abstracts, J Equine Vet Sci 32:510.
35) Robertshaw, D., and J.E. Vercoe. 1980. Scrotal thermoregulation of the bull (Bos sp.). *Aust. J. Agric. Res.* 31:401-407.
36) Setchell B.P. 1970 Testicular blood supply, lymphatic drainage and secretion of fluid. In The Testis pp101-239 Eds AD Johnson, WR Gomes and NL Vandermark, Academic Press, New York.
37) Setchell, B.P. 1998. The Parkes Lecture Heat and the testis. *J Reprod Fert*. 114:179-194.
38) Smaga, A., T. Paszkowski, S. Wozniak, R. Walczak. 2003. Analysis of physiologic and abnormal pictures of uterine cervix by means of infrared thermography. *Ginekol Pol* 74(9):847-54.
39) Stelletta, C., A. Stefani, I. Marion, Y. Bellicanta, C. Cannizzo and S. Romagnoli. 2009. Effect of GnRH test on scrotal surface temperature in Alpaca. 2nd Conference of the International Society of Camelid Research and Development, 11th-14th March, Djerba, Tunisia.
40) Stelletta, C., G. Stradaioli, M. Gianesella, I.M. Mayorga Munoz, M. Morgante. 2006. Studio termografico della area perivulvare in pecore in fase estrale ed in fase anaestrale. In: XVII Congresso Sipaoc. Lamezia Terme (Cz), 25/28 Ottobre.
41) Stelletta, Calogero, Matteo Gianesella, Juri Vencato, Enrico Fiore and Massimo Morgante. 2012. Thermographic Applications in Veterinary Medicine, Infrared

Thermography, Dr. Raghu V Prakash (Ed.), ISBN: 978-953-51-0242-7, InTech, DOI: 10.5772/29135. Available from: http://www.intechopen.com/books/infrared-thermography/thermographic-applications-in-veterinary-medicine
42) Vencato, J., L. Cestaro, I. Vazzana, G. Carrer, E. Carlo, S. Dara, and C. Stelletta. 2012. Thermographic monitoring of scrotal surface during GnRH test in young bulls with low semen quality. Proc. 17th International Congress on Animal Reproduction (ICAR) 29 July-2 August, Vancouver, Canada.

Table 1. Values of thermographic, ultrasonographic and electrical parameters during the periovulatory period in mare

	T1	T2	T3
TMinP	28.6±0.80	28.84±0.32	27.72±0.54
TMaxP	34.2±0.38 [ab]	34.41±0.22 [a]	33.43±0.13 [b]
TMP	31,98±0.51 [a]	31.54±0,15 [ab]	30.91±0.21 [b]
TMinV	27.95±0.22	27.17±0.61	26.85±0.28
TMaxV	33.72±0.46 [ab]	34.12±0.18 [a]	33.14±0.14 [b]
TMV	31.40±0.30 [a]	31.31±0.19 [a]	30.31±0.20 [b]
TMDt	0.58±0.23	0.23±0.16	0.60±0.19
θFM	4.31±0.28 [a]	4.72±0.21 [a]	1.10±0.13 [b]
EPF	2.30±0.12 [a]	2.45±0.11 [a]	1.0±0.00 [b]
CF	1.58±0.07 [a]	1.79±0.04 [b]	1.0±0.00 [c]
EU	1.50±0.29 [a]	1.39±0.14 [a]	0.92±0.05 [b]
ER	320.00±21.99	391.11±23.12	366.67±22.11
P4	0.2±0.00	0.23±0.01	0.31±0.03

Different letters among groups indicate a significant difference (P<0,05) Perivulvar Minimum temperature (PMinT); Perivulvar Maximum Temperature (PMaxT); Perivulvar Mean Temperature (PMT); Vulvar Minimum Temperature (VMinT); Vulvar Maximum Temperature (VMaxT); Vulavr mean Temperature (VMT); Delta mean temperature Perivulvar-Vulvar (ΔPVMT); Diameter of Greater Follicle (DGF); Follicle concistency (CF) grade 1= firm, grade 2=soft, Echotexture Follicular Wall(EFW)grado 1= anecogenic, grade 2= medium ecogenicity, grade 3=ecogenic; Uterine edema (UE) grade 1=mild, 2=moderate, 3=heavy; Electrical impedance (EI); Progesterone (PG); T1(DGF>3cm); T2 (follicle growing); T3 (ovulation).

Figure 1: Thermographic images of vulva and perivulvar regions taken during administration of the second dose of GnRH (a) and AI (b) in a Ovsynch protocol. The measuring scale indicates the color relating to temperature (°C).

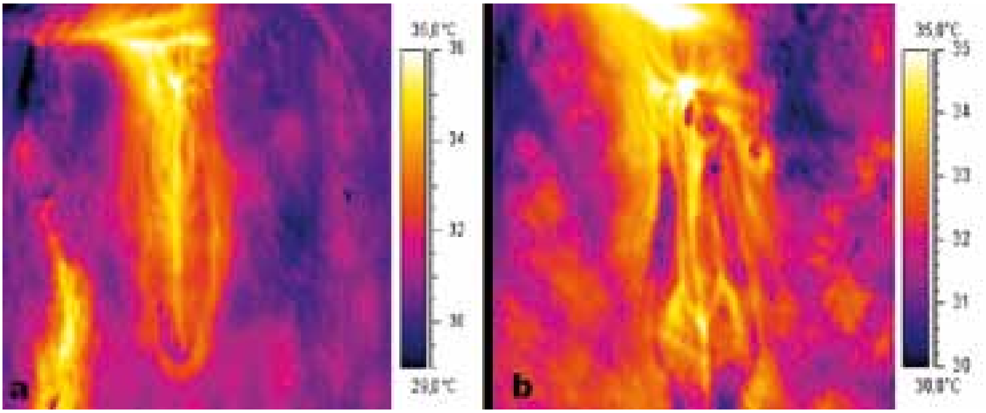


Figure 2. Ewes vulvar thermograms during a synchronization protocol.

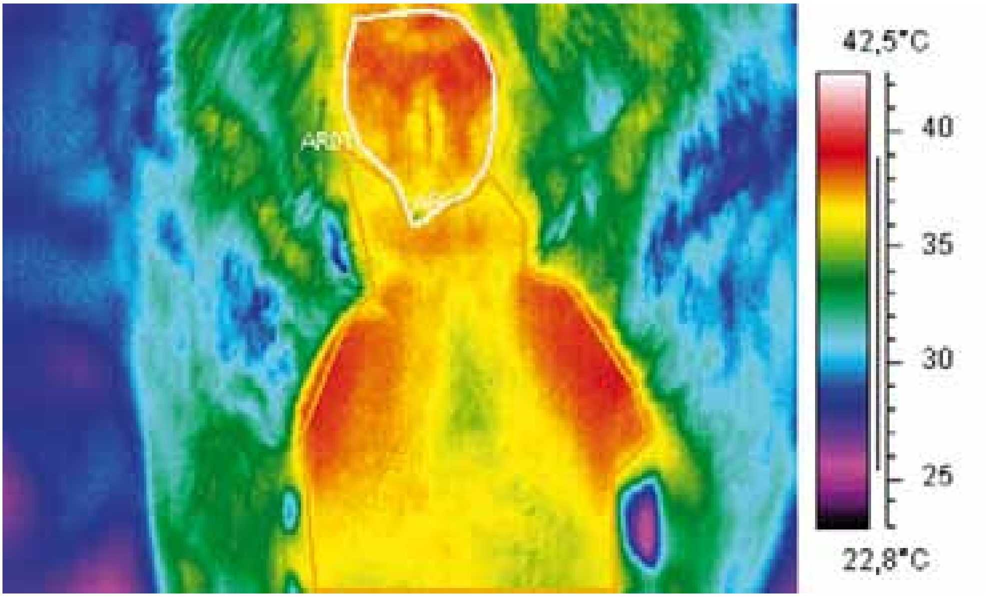


Figure 3. Normal scrotal thermogram in bull.

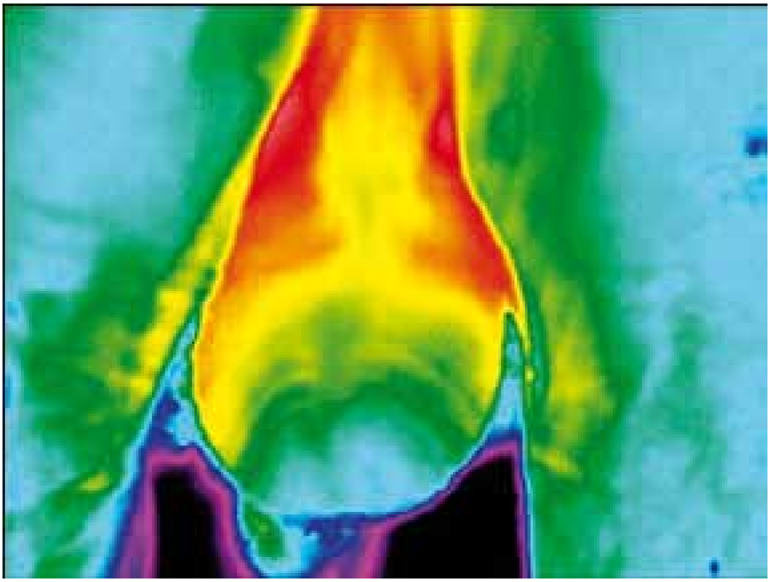
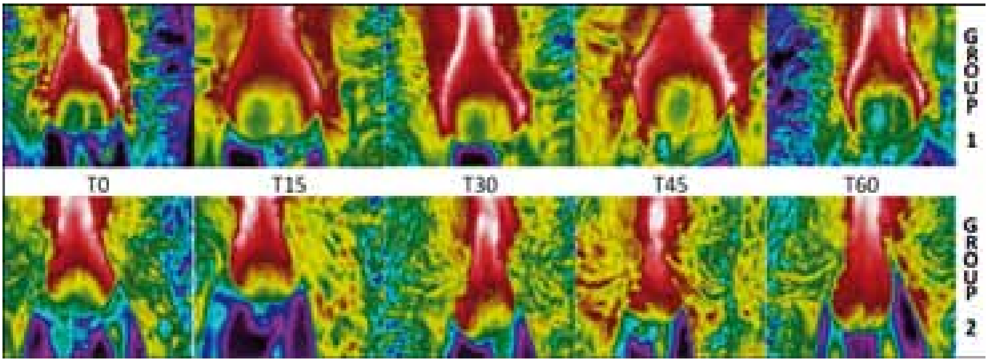
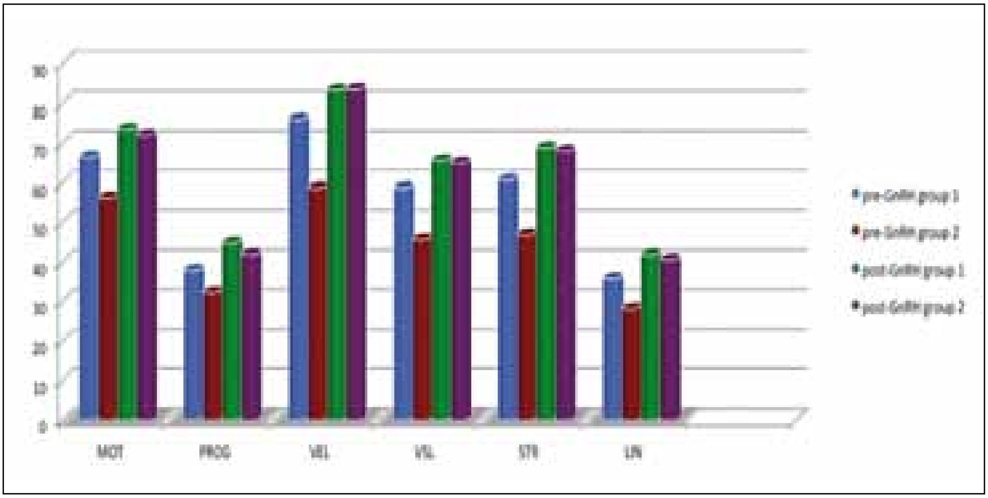


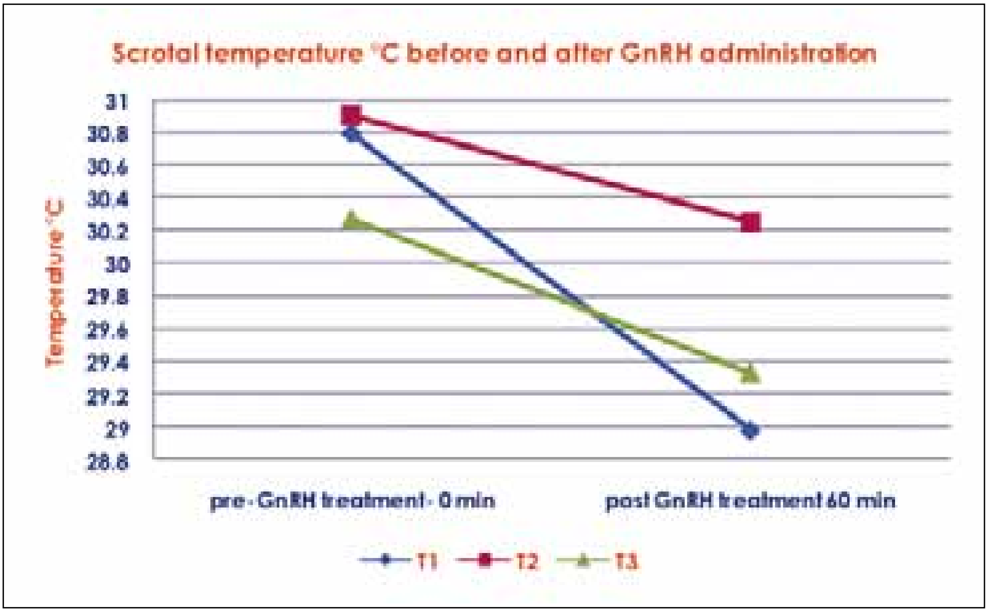
Figure 4. Scrotal surface temperature monitoring after GnRH administration in bull (Vencato et al., 2012). Group 1=decreased temperature after 60; Group 2=increased temperature after 60 minutes.



Graphic 1: Sperm quality in groups with different thermal response to GnRH administration (Vencato et al., 2012) Group 1= decreased temperature after 60 minutes; Group 2= increased temperature after 60 minutes.



Graphic 2: Scrotal surface temperature in alpaca before and after GnRH administration (Stelletta et al., 2009).



VETERINARY DIAGNOSTIC IMAGING

SILVIA MARCHES, MELANIA MOIOLI, MAURO DI GIANCAMILLO, FABIO LUZI

VESPA Department – Università degli Studi di Milano, Italy

ABSTRACT

In Veterinary Medicine, Diagnostic Imaging includes radiology, ultrasonography, computed tomography, magnetic resonance and nuclear medicine. Infrared technology (IR) is a new non-destructive and no-contact technique: it gives a real time dynamic visual map of gradients of temperatures on a body area. Thermography is useful, both in humans and in animals, for the early diagnosis of pathologies characterized by microcirculation changes, inflammation, metabolism and thermoregulatory systems alterations. This technique has a good sensitivity, can be used without anesthesia and it's not a source of stress for the patient. It can be very effective in locating and evaluating the extent of areas affected by different pathologies, but only provides generic and non-specific information. For these reasons IR can be considered a complementary aid, in combination with a complete clinical examination and other diagnostic techniques. Infrared Thermography technique is possible to apply for mass screening, as for farm animals. In fact, an important aspect of this technology which has a strong impact on welfare is its preventive nature: IRT camera is able to discover changes that have not yet caused clinical signs in apparently healthy subjects. Thermography allows to control a large number of animals in a short time and animals chosen may be subsequently subjected to additional controls with other techniques.

Key words

Veterinary Medicine, non-invasive technique, diagnostic imaging, radiography, ultrasound, computed tomography, magnetic resonance imaging, infrared thermography.

Diagnostic imaging or medical imaging uses electromagnetic radiation to produce images of internal body structures for diagnosis. In veterinary medicine, diagnostic imaging includes radiology, ultrasonography, computed tomography, magnetic resonance and nuclear medicine and a complete series of two and three dimensional images of the body and/or single structures can be obtained. All these techniques can be considered non invasive, and most of them are commonly utilized in the diagnosis of diseases in animals.

Radiographs are produced by an X-ray beam passing through animal's body.

In film-screen radiography, imaging procedure uses silver-impregnated films. A capture device, comprising a screen of light-emitting phosphors, held tightly in a light-proof cassette, converts the x-ray beam into visible light, which then creates an image. The cassette, contained the exposed film, is then taken into a darkroom for developing in chemical tanks or is developed by an automatic film processor and finally the image appears on the film.

Medical Imaging	Electromagnetic Radiation	Cost	Time (Duration of procedure)
Radiology	x-rays	low	low
Ultrasonography	Ultrasound	medium	low
Computed Tomography	x-rays	high	high
Magnetic Resonance Imaging	Magnetic fields and ultrasound	high	high
Nuclear Medicine	γ-rays	high	medium

Film-screen radiography has been replaced recently by computed radiography (CR) and direct radiography (DR). Computerised radiography uses the same equipment as conventional radiography except that in place of a film-screen system to create the image, an imaging plate (IP) made of photostimulable phosphor is used. The procedures are the same as for radiology, and a special cassette, contained the IP, is placed under the animal's body and the x-ray exposure is made. After x-ray exposure, the imaging plate is run through a special laser scanner, or CR reader, that reads and digitizes the image. The digital image can then be viewed and enhanced using software that has functions very similar to other conventional digital image-processing software, such as contrast, brightness, filtration and zoom.

In Direct Radiography, the X-ray beam strikes a plate of sensors (flat panel detectors) that convert the signals generated into digital information, which is transmitted and converted into an image displayed on a computer screen.

Independently from the technology utilized, X-rays can have the same interaction with matter. There are three methods of interaction:

- No interaction: X-rays pass completely through tissue and into the image recording device;
- Complete absorption: X-rays are completely absorbed by the tissue;
- Partial absorption with scatter: scattering involves a partial transfer of energy to tissue, with the resulting scattered X-ray having less energy and a different trajectory. Scattered radiation tends to a degradation of image quality and is the primary source of radiation exposure to operator and staff.

In diagnostic radiology there are two important interactions with matter, photoelectric absorption and Compton scattering. In the photoelectric effect the photon is totally absorbed by matter and it is not scattered. In Compton scattering, scattered radiation produces a uniform optical density on the radiograph that reduces image contrast. The photoelectric effect provides information because it allows imaging of anatomical structures with high X-ray absorption characteristics, radiopaque structures, and tissues with a high atomic number or tissues with high mass density. Compton scattering contributes no useful information: photons are partially absorbed, and X-rays emerge from the interaction travelling in a different direction (sometimes with less energy). Compton scattering creates an image "*fog*" that does not provide any diagnostic information. It is *independent* of the atomic number of tissue. The probability of Compton scattering is the same for bone atoms and for soft tissue atoms. The probability for Compton scattering is more *dependent* on kVp (the maximum *voltage* applied across an *X-ray tube*. It determines the *kinetic energy* of the *electrons* accelerated in the X-ray tube and the peak energy of the *X-ray emission spectrum*).

Image formation is due to differential absorption, the difference between those X-rays absorbed and those transmitted to the film-screen system (or plate in CR or detector in DR). Compton scatter provides no useful information, photoelectric absorption produces the light areas on the image and transmitted X-rays produce the grey/dark areas on the image. Differential absorption increases as kVp is reduced and the probability of radiation interaction is a function of tissue electron density/atomic number, tissue thickness/density and the energy of the X-rays (kVp). Dense matter, such as bones and contrast media, attenuate the X-rays more than less dense matter (soft tissues). The different rate of attenuation provides the contrast (gray scale) necessary to form an image. Tissues that do not allow the transmission of X-rays through the material are radio-opaque, absorbing most of the X-rays and thus appearing "light" on the radiograph. Such materials include bone, metal and any contrast medium. Radiolucent are the tissues that allow free travel of X-ray energy through the material (for example air in the lobes of the lungs) are termed radiolucent and only absorb a small amount of the X-rays and thus appear black on a radiograph. Between the two extremes of black and light there is the dynamic range (gray scale) in which tissues can assume different levels of gray according to their own linear attenuation coefficient (LAC) or absorption coefficient. Every tissue will be different to each other, for example liver has a higher LAC than fat, but a lower LAC than bone and fat has a higher LAC than lung. So liver, fat and lung will be radiolucent with regard to bone, but liver will be more radiopaque than fat, and fat will be more radiopaque than lung.

Recently, film-screen radiography had been replaced by digital radiography (CR or DR). A digital radiographic image is formed as an electronic image displayed on a grid laid out in rows and columns called an image matrix. An image can be made of thousands, preferably millions of these small cells. Each cell in the image matrix is called a picture element, or pixel. With digital imaging, each pixel will have a numerical value that determines the brightness (density) or other details of the cell. Each box has its own dynamic range of values according to the number of bytes of processing; this is the gray-scale range. For one byte there are 256 possible values for the density of each pixel, and with 16 bit processing there are 65,535 possible densities which any cell can have. These densities can be correlated with the energy of the photons that strike phosphors in the recording medium from which the image will be reconstructed. So, if we use for example, 16 bit processing, and millions of cells in our matrix, we can have a very high range for exposure and image details.

A digital computed radiography image matrix is at least 512 x 512 which contains 262,144 pixels. This pixel size is comparable to analog screen-film imaging. Advanced CR systems can produce images using a 1024 x 1024 matrix or greater which will contain more information than a comparable analog image.

Pixel size alone does not determine the detail of an image and the range of values each pixel may have is also very important, as well as the number of pixels itself. The gray scale is a function of both the hardware and software in converting the image into digital form, and, for example, the dynamic range of an 8-bit processor is 0-256 densities. With all other factors being equal, 8-bit processing will have less gray scale resolution than an image produced by 9-bit or 10-bit processing. The dynamic range is expressed in bits, meaning an 8-bit image will have less clarity and gray scale than a 10-bit or 12-bit processor. Many of today's computed radiography and direct digital radiography images use 16-bit processing or higher.

Simple radiography was the only imaging approach available during the first 50 years of veterinary radiology. Due to its availability, speed, and lower costs compared to other methods, radiography is often the first-line test of choice in radiological diagnosis. Also, despite the large amount of data in CT scans, MR scans and other digital-based imaging, there are many diseases in which the classic diagnosis is obtained by plain radiographs (Dennis et al., 2010; Thrall, 2012).

Computed tomography (CT scan) is a diagnostic imaging technique that uses a computer-processed collimated X-ray beam to produce cross-sectional (tomographic or transverse or slice) images of specific areas of an animal's body. The first CT scan was invented by Sir

Godfrey Hounsfield at EMI Central Research Laboratory, in 1972, funded from the profits of the distribution rights of music by the Beatles. In 1979, Sir Hounsfield and Alan McLeod McCormick shared the Nobel Prize for medicine for the invention of CT imaging.

CT imaging uses X-rays in conjunction with computing algorithms to image the body. An X-ray tube opposite an X-ray detector in a ring shaped assembly rotates around the animals producing a cross sectional image. The CT image is only acquired on the transverse plane, and other views, such as dorsal and sagittal images, are produced by computer reconstruction, called multiplanar reconstruction (MPR). CT imaging completely eliminates the superimposition of images of structures outside the area of interest, typical of two-dimensional radiography. One of the main advantages of CT is the inherent high-contrast resolution of CT, where differences between tissues that differ in physical density by less than 1% can be distinguished. Recent developments in multi-detector CT technology now allow its use in veterinary medicine which introduces improvements to diagnostic capabilities previously outside the realm of CT scanning.

In CT the term voxel has had to be introduced. Pixel is term common used in digital radiography, but it is a two dimensional unit based on the matrix size and the field of view. Voxel (volume element) is a three-dimensional unit, because every slice of the body has an own thickness.

Every voxel represents the radiodensity of the tissue. Radiodensity can also be quantified in accordance with the Hounsfield scale (gray scale in CT or CT scale or CT number).

Tissue	Hounsfield Unit (mean)
Bone	range from +400 up to +1000 or more
Cartilage	+100/+150
Soft tissue	+ 20/+70
Distilled water	0
Fat	- 110/-70
Air	-1000

Modern technology offers isotropic resolution that allows the CT software to display the body volume acquired in an alternative manner. This technology increases the resolution on multiplanar reconstruction and it is possible to explore a large body volume in a short time, which is useful in angiography. Maximum-intensity projection (MIP reconstruction) enhances areas of high radiodensity and it is very useful in angiographic studies. Others applications of CT are 3D rendering techniques. Surface rendering allows three-dimensional models to be obtained to represent each anatomical component such as bone and cartilage, using specific processing algorithms. However, surface rendering only allows a representation of the surface of the object. Volume rendering instead allows a better representation of the acquired volume by means of transparency models and color applications. Imaging segmentation, manual or automatic, is useful when is not possible to use volume-rendering parameters and unwanted structures need to be removed (Dal Pozzo, 1999; Pozzi Mucelli, 1996; Schwartz and Saunders, 2011).

Ultrasonography uses ultrasound rather than electromagnetic radiation and it is based on echoes produced by an ultrasound beam travelling through organs or tissues.

A transducer in contact with the skin converts an electric current into sound waves and, as they encounter tissue interfaces of different acoustic impedance (the product of a tissue's physical density and sound velocity) a reflection is generated. Only the waves which encounter a medium with an angle of incidence perpendicular to the reflector return to the transducer, containing diagnostic information for the creation of an image.

Different values of acoustic impedance determine how much of the sound wave is reflected and how much is transmitted into the second tissue.

Ultrasound that is not reflected continues through the tissues with a lower intensity and with a different angle.

Liquids transmit sound waves very well and cause no reflection, so there are no return echoes (black image). Gas and bone generate maximum reflection with the production of intense echoes (white image), while the soft tissues produce grey scale images (Dennis et al., 2010; Penninck and D'Anjou, 2008).

	TERMINOLOGY
Radiography	Radio-opaque / Radiolucent
Ultrasound	Hyperechoic / Hypoechoic
Computed Tomography	Hyperdense / Hypodense
Magnetic Resonance Imaging	Hyperintense / Hypointense
Nuclear Medicine	Hot spot / Cold spot

Magnetic Resonance Imaging (MRI) provides digital tomographic images using magnetic fields and radio frequency without ionizing radiation. MRI uses nuclear magnetic resonance to produce high resolution images. Body tissue contain a great deal of water, and hence *protons* (^{1}H nuclei). Protons may be aligned by exposure to a very strong magnetic field. Each water molecule has two *hydrogen nuclei* or *protons*. When a live subject is placed inside the powerful *magnetic field* of an MRI scanner, the average *magnetic moment* of many protons becomes aligned with the direction of the field. A radio frequency current is briefly turned on, producing a varying *electromagnetic field*. This electromagnetic field employed is at the "resonant *frequency*", to ensure maximum absorption of energy which causes the *spin* of the protons to "flip". After the electromagnetic field is turned off, the spins of the protons return to thermodynamic equilibrium and the bulk magnetization becomes re-aligned with the static magnetic field. During this relaxation, a radio frequency signal (*electromagnetic radiation* in the RF range) is generated, which can be measured with receiver coils and the image (in a transverse or longitudinal plane) is generated from these signals. MRI is a multiparametric, multiplanar technique which allows the acquisition of images on sagittal, dorsal and transverse planes without moving the patient. The contrast of the image can be changed in a predetermined and controllable way.

The remote measurement is the quantity of magnetization on the transversal plane and the attenuation rate (called T2 relaxation) and the spin (called T1 relaxation). The emitted signals can be collected for conversion into shades of grey in the MR image by placing a receiver coil close to the patient. The mode and duration of the electromagnetic pulses determines the intensity of the emitted signal and the contrast will be different because of the density of protons and of transversal and longitudinal relaxations. This depends on different biophysical properties allowing the characterization of normal and pathological tissues (Elliot and Skerrit, 2010; Gavin and Bagley, 2009; Westbrook et al., 2011).

Nuclear Medicine allows morpho-functional data to be obtained both *in vivo* and *in vitro using* radioactive isotopes (radionuclides). Applications for this technique include diagnostic investigations on patients given radioactive medication (mainly intravenous, but even intra-arterial administration, or through inhalation etc.) and measuring the resultant radiation. Another application is in laboratory investigations, which test radioactive activities of biological samples injected *in vivo* and/or *in vitro*.

Information from Nuclear Medicine comes from images and quantitative data. Nuclear Medicine is nowadays the only technique in which the image of an organ or tissue shows its normal or pathological functionality. This feature of nuclear medicine sciences allows the early detection of pathologies since diseases cause biochemical and functional alterations. Every application of nuclear medicine requires the body to assimilate, transport, spread, accumulate and remove radionuclides. Scintigraphy is the production of a two-dimensional image of the distribution of radioactivity in tissues following the internal administration of a radiopharmaceutical imaging agent.

Nuclear-medicine studies are best performed by Positron Emission Tomography (PET). It employs positron emission from elements, which are isotopes of the same atoms of organic molecules without changing the biochemical properties of organic molecules. Unfortunately Nuclear Medicine is still unpopular in Italy, because of costs and of the rigid regulation of implants and the management of radioactive medicaments. However, PET is widely used to study the skeletal system in horses and in small animal oncology (Moretti et al., 1993).

Infrared *(*IR*)* technology has developed recently in diagnostic imaging and it can be regarded as a non-destructive technique because it doesn't require any injury or mechanical effect on the surface of the patient in order to reaching diagnosis. It gives, in real time, a dynamic visual map of temperature gradients on a body surface, producing images and enabling an analysis of the surface body temperature.

The thermal information acquired reflects tissue metabolism and blood circulation, which has a key role in the regulation of body temperature and is adjusted by the nervous and the endocrine systems.

It is possible to identify a change in blood circulation change due to a pathological stimulus or to an alteration in the patient's physiological state. Limited areas of hyperthermia may be associated with an increase of arterial flow or with venous stasis, while hypothermia areas can indicate a decrease in blood perfusion in those areas, or a reduction in vasomotor tone. Degenerative processes, fibrosis, involution of parenchymatous organs and circulatory problems cause a decrease of local temperature in affected areas because of the reduced blood flow. An excessive blood flow in a traumatized areas causes an increase of temperature (Gatto,2010).

Pyroscan is the first tool used in 1942 to diagnose inflammatory joints diseases. (Ring, 2004) More innovative tools developed between 1960 and 1970 allowed the production of color images. Nowadays, thermographs allow the monitoring of temperature fluctuations in real time and to have quantitative and functional investigations of dynamic temperature (Kastberger, 2003).

There are several studies and applications of thermography in medicine, which is used as a collateral diagnostic tool for the early and non-invasive diagnosis of breast cancer (Golab-Lipinska et al., 2004; Wright and McGechan, 2003; Head et al., 1993; Isard et al., 1988; Nyirjesy, 1982; Gautherie and Gros, 1980), malignant melanoma (Tapernoux and Hessler, 1977), in the study of head and neck region tumors (Misiolek et al., 1999) and pathologies affecting the thyroid gland (Karmadin and Kuzmichev, 1983). Female (Shah et al., 1984,; Smaga et al., 2003) and male (Tucker, 2000; Amiel et al., 1976; Coppola et al., 1984; Coulter et al., 1988) reproductive system thermography have been studied. More recently infrared technology has been used to monitor temperature variations that occur over the entire maternal abdominal wall during gestation (Beinder et al., 1990), labor and birth (Yang et al., 1990).

There are several studies in cardiovascular medicine and surgery, since vascular diseases can be well analyzed through infrared (Gatto, 2010). Thermography is useful for the diagnosis of deep venous thrombosis (Holmgren et al., 1988; Bergqvist et al., 1977), for the early detection of ischemic region perfusion dysfunctions (Spence et al., 1984) and in studies relating to transplanted organs (Kopsa et al., 1979), brain function (Karpman et al., 1972), the heart (Adachi et al., 1987) and coronary conditions (Robicek et al., 1978; Cirino, 2004). Thermography can also be used in postoperative control to evaluate surgery efficacy and postoperative reperfusion (Skliarenko and Zakalinskii, 1986; Merla et al., 2002). The acute effects of cigarette smoke on increasing blood pressure and reducing superficial temperature have been also evaluated (Di Carlo and Ippolito, 2003). This approach has limited diagnostic value (So et al., 1998) and a low prognostic value for neuromuscular diseases, radiculopathies and neuropathies (Ming et al., 2005).

Infrared thermography has been also used as a mass-screening tool to detect fever in patients suspected to have severe acute respiratory syndrome (SARS) (Chiu and Lin, 2005) and in experimental studies on rats to identify a pneumothorax (Rich et al., 2004). Other studies have been conducted on diabetic people (Stess et al., 1986; Sun et al., 2005; Mitchell et al., 1989) and patients with spine pathologies, such as spinal cord lesions, scoliosis and disc protrusions (Sherman et al., 1987; Milano et al., 1982).

In veterinary medicine infrared thermography studies and applications are much more limited, especially in Small Animal Clinic. In equine practice thermography is mainly used for the diagnosis of muscle and skeletal diseases, as well as for ligaments, tendons (Marr et al., 1993), joints (Turner, 1996) and muscles pathologies. Thermography has shown to be, in combination with other diagnostic tools (such as radiology, ultrasonography and scintigraphy) and in combination with a complete clinical examination, a practical aid in the clinical assessment of lameness in horses (Turner, 1991; Eddy, 2001; Weil, 1998; Stashak, 1987; Denoix, 1994; Embaby et al., 2002). It allows early detection of orthopaedic problems, such as navicular disease (Turner et al., 1983), stress fractures and tendinitis (Turner, 1991,; Marr et al., 1993) and several other equine foot disorders such as laminitis, abscesses and pododermatitis (Turner, 1996), even without clinical symptoms and radiological abnormalities being evident. Another possible use of this infrared technology is in the detection of muscular disorders in the backs of horses (Schweinitz, 1999; Shamaa and Gohar, 2002) and for pregnancy diagnosis or confirmation (Bowers et al., 2004).

In bovines, clinical infrared thermography is mainly used for the early detection of lameness (Nikkhah et al., 2005), mastitis (Scott et al., 2000; Berry et al., 2003) and oestrus (Hurnik et al., 1985). It has been also used as a non-invasive method for the early identification of calves suffering from bovine viral diarrhoea (BVD) (Schaefer et al., 2003), of cattle with bovine respiratory disease (BRD) (Schaefer et al., 2006) and for the evaluation of scrotum temperature as an index of fertility in bulls (Lunstra and Coulter, 1997).

In small animals, there are a few studies on healthy dogs limbs (Loughin and Marino, 2007), particularly on the stifle joint (Infernuso et al., 2010).

Therefore, both in humans and animals, the thermographic technique is useful for those pathologies characterized by microcirculation changes, inflammation, and changes in metabolism and thermoregulatory systems. It provides good sensitivity and can be used without anesthesia without imposing any stress on the animal.

However, thermography, using skin temperature as an indicator of an underlying disease process, can be very effective in locating affected areas and in evaluating the extent of the disease process. However, it only provides generic and non-specific information. Moreover, the spatial resolution of the system is not particularly high and is inevitably affected by the distance between the camera and the anatomical region being examined. In small animal pathology and clinical studies it is important to consider the limitations of this diagnostic technique, as well as for all the other imaging techniques.

From the limited literature available, it is not easy to identify specific applications for thermography in small animals. However, based on our knowledge from studies with humans, we can assume that thermography can be used in the study of skin and its appendages. However, it is important to highlight that the surface covering of the animal (fur, feathers, etc.) may interfere with correct temperature measurement. Because of this, hair removal is necessary which may not always be acceptable to pet owners for a simple diagnostic procedure.

Another interesting field for the application of thermography is in orthopedics, particularly in the study of small joints, where it could also be used as a general screening to evaluate lameness in dogs with chronic degenerative arthropathy. Even if the radiographic examination remains essential to assess a correct diagnosis, thermography could play a complementary role for the temporal monitoring of joints. For example, a therapeutic treatment effectiveness can be indirectly monitored by measuring the temperature changes of an osteoarthrosic joint.

There is insufficient published data to make a judgment on the use of thermography for studying large body cavities. Literature shows the possible use of thermography in studying the female reproductive system both in normal and pathological conditions, although ultrasonography, supported by radiography, computed tomography X-ray and/or MRI, is generally considered the first choice technique for abdomen study.

The authors do not know of any published reports on the use of thermography in studying the cardiovascular system, lower respiratory tract or nervous system of small animals.

Although applications similar to human medicine can be assumed, other imaging techniques, such as echo-color-doppler, subtractive angiography, angio/cardio CT and angio/cardio MRI play a key role in cardiovascular clinical diagnostics.. The same considerations may apply to the thorax, where diagnostic radiography (digital, direct and indirect) and X-ray computed tomography techniques are to be considered elective. Finally, CT and especially MRI, due to its peculiar tissue characterization aspects, are the preferred and elective techniques for the nervous system.

Thermography may have a possible application in the mass screening of farm animals. An important aspect of this technology, which has a strong impact on welfare, is its use in preventive measures. An infra red thermal camera is able to discover changes that have not yet caused clinical signs in apparently healthy subjects. Thermography thus allows the remote inspection of a large number of animals in a short time and animals identified as needing further examination may be subjected to additional controls with other techniques.

This feature is very useful for pathologies of the feet in cattle and horses, where lameness is often due to a combination of factors and creates asymmetries and fatigues in some muscles which can be identified with the help of thermography (Milazzo et al., 2002; Gerken and Barow, 1998; Schefer et al., 1989; Svartberg, 2005;Turner, 1991).With regard to dairy cows (Figure 9) the thermographic technique has proved particularly useful in the early detection of foot diseases (Knizkova et al., 2007), allowing early intervention and improving animal welfare, as well as reducing the negative economic impact of these conditions for the farmer. Thermography can be useful even with the poor performance syndrome in sport horses (Figure 10), where thermographic videos recorded during exercise allow the clinician to visualize the heating of the different muscle areas and monitor the proper vascularization of tissues, as well as identifying abnormal responses due, for example, to disease. Particularly interesting is the possibility of using the thermal camera to monitor the skin temperature of animals during transport, a condition considered to be very stressful (Figure 11).

In the field of veterinary applications, active thermography (or functional imaging), allows the development of dynamic sequences of thermal images with special algorithms, which could not be obtained from single frame images.

Possibly the major limitation to the application of thermography in the animal and biological field, lies in the ease of use and in its extreme sensitivity. In fact, thermal images of an animal often show thermal anomalies which are not directly related to its physio-pathological state, but due to extraneous factors such as environmental conditions or those of measurement, or to individual variability. Any small variations in environmental temperature, or other sources of infra-red radiation, can be considered as influencing the true temperature of the animal skin.

Further, each animal species presents unique technical challenges, due to the species-specific differences (size, presence of fur, behavioral characteristics, housing, etc..), that can affect the success of the measures.

REFERENCES

1) Adachi, H., Becker, L.C., Ambrosio, G., Tekeda, K., Dipaula, A.F., Baumgartmer, W.A., Borkon, A.M. and Reitz, B.A. (1987) Assessment of myocardial blood flow by real-time infrared imaging. *J. Surg. Res.* 43, 94-102.
2) Amiel, J.P., Vignalou, L., Tricoire, J., Jamain, B. and, Ravina, J.H. (1976) Thermography of the testicle. Preliminary study. *J. Ginecol. Obstet. Biol. Reprod. (Paris)* 5, 917-923.
3) Beinder, E., Huch, A. and Huch, R.J. (1990) J. Peripheral skin temperature and microcirculatory reactivity during pregnancy. A study with thermography. (1990) *Perinat. Med.* 18, 383-90.
4) Bergqvist, D., Efsing, H.O. and Hallbook, T. (1977) Thermography: A non-invasive method for diagnosis of deep venous throbosis. *Arch. Surg.* 112, 600-604.
5) Berry, R.J., Kennedy, A.D., Scott, S.L., Kyle, B.L. and Schaefer, A.L. (2003)Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: potential for mastitis detection. *Canadian Journal of Animal Science* 83, 687-693.
6) Bowers, S., Gandy, S., Anderson, B., Ryan, P. and Willard, S. (2004) Assessment of pregnancy in the mare using digital infrared Thermography. *American Society of Animal Science/American Society of Dairy Science*, . *J. Anim. Sci.* 82 (Suppl. 1):371.
7) Chiu, W.T., Lin, P.W., Chiou, H.Y., Lee, W.S., Lee, C.N., Yang, Y.Y., Lee, H.M., Hsieh, M.S., Hu, C.J., Ho, Y.S., Deng, W.P. and Hsu, C.Y. (2005) Infrared thermography to mass-screen suspected SARS patients with fever. *Asia Pac J Public Health* 17, 26-8.
8) Cirino, F. (2004) Sviluppo di modelli ed algoritmi per l'analisi del flusso nella circolazione coronaria attraverso immagini di termografia intraoperatoria ad alta risoluzione. Tesi di laurea in Ingegneria elettronica. *Università di Pisa*, 2004.
9) Coppola, V., Verrengia, D., Rossi, R. and, Gallo, S. (1984) Telethermography: application to problems of infertility. *J. Reprod. Fertil.* 84, 417-423.
10) Coulter, G.H., Senger, P.L. and Bailey, D.R. (1988) Relationship of scrotal surface temperature measured by infrared thermography to subcutaneous and deep testicular temperature in the ram. *J. Reprod. Fertil* 84, 417-423.
11) Dal Pozzo, G. (1999) Compendio di tomografia computerizzata e TC spirale. *UTET*.
12) Dennis, R., Kirberger, R.M., Barr, F. and Wrigley, R.H. (2010) Handbook of Small Animal Radiology and Ultrasound. *Saunders*, 2nd Edition.
13) Denoix, J.M. (1994) Diagnostic techniques for identification and documentation of tendon and ligament injuries. *Veterinary Clinics of North America: Equine Practice* 10, 365-407.
14) Di Carlo, A. and Ippolito, F. (2003) Early effects of cigarette smoking in hypertensive and normotensive subjects. An ambulatory blood pressure and thermographic study. *Minerva Cardioangiol.* 51, 387-93.
15) Eddy, A.L., van Hoogmoed, L.M. and Snyder, J.R. (2001) Review: The role of thermography in the management of equine lameness. *Vet. J.* 162, 172- 181.
16) Elliot, I. and Skerrit, G. (2010) Handbook of Small animal MRI. *Wiley-Blackwell*.
17) Embaby, S., Shamaa, A.A. and Gohar, H.M. (2002) Clinical assessment of thermography as a diagnostic and prognostic tool in horse practice. In: Proceedings of Inflammation, Orlando, USA, 30 – 36.
18) Gatto, M. (2010), *La termografia in medicina veterinaria "something new about something old": il suo valore diagnostico nel benessere animale*. PhD Thesis, University of Padova.
19) Gautherie, M. and Gros, C.M. (1980) Breast thermography and cancer risk prediction. *Cancer* 45, 51-56.
20) Gautheire, M., Heahnel, P., Walter, P.J. and Keith, L.G. (1982) Thermovascular changes associated with in situ and minimal breast cancers. Result of an ongoing prospective study after four years. *J. Reprod. Med.* 32, 833-842.

21) Gerken, M. and Barow, U. (1998) Methodical investigation into thermoregulation in suckler cows under field conditions. *Proc. 49th Annual Meeting of EAAP, Warsaw, Poland.* 179, 18.
22) Golab-Lipinska, M.V., Jakubowska, T., Wysocki, M., Kaluzna, K. and Peszynski-Drews, C. (2004) Thermography in the early detection of breast cancer-our own experiences. *Wiad Lek* 57, 87-90.
23) Head, J.F., Wang, F. and Elliott, R.L. (1993) Breast thermography is a noninvasive prognostic procedure that predicts tumor growth rate in breast cancer patients. *Ann N Y Acad Sci.* 698, 153-8.
24) Holmgren, K., Andersson, G., Fagrell, B., Johnsson, H., Lofsjogard-Nillson, E. and Zetterquist, . (1988) One-year sequential follow-up of venous emptying rate and leg temperature profiles after acute deep vein thrombosis. *Acta Med. Scand.* 224, 577-582.
25) Hurnik, J.F., Webster, A.B., and DeBoer, S. (1985) An investigation of skin temperature differentials in relation to estrus in dairy cattle using a thermal infrared scanning technique. *Journal of Animal Science* 61, 1095-1102.
26) Infernuso, T., Loughin, C.A., Marino, D.J., Umbaugh, S.E. and Solt, P.S. (2010) Thermal imaging of normal and cranial cruciate ligament-deficient stifles in dogs. *Veterinary Surgery* 39, 410-417
27) Isard, H.J., Sweitzer, C.J. andEdelstien, G.R. (1988) Breast thermography: a prognostic indicator for breast cancer survival. *Cancer.* 62, 484-488.
28) Karmardin, L.N. and Kuzmikev, A.S. (1983) Thermography in the differential diagnosis of nodular goiter and thyroid cancer. *Vestn. Khir*, 70-74.
29) Karpman, H.L., Kalb, I.M. and Sheppard, J.J. (1972) The use of thermography in a health care system for stroke. *Geriatrics* 27, 96-105.
30) Kastberger, G. and Stachl, R. (2003) Infrared imaging technology and biological applications. *Behav Res Methods Instrum Comput.* 35, 429-39.
31) Knizkova, I., Kunc, P., Guzdil, G.A.K., Pinar, Y. and Selvi, K.C. (2007) Applications of infrared thermography in animal production. *J. of Fac. Agric.* 22, 329-336.
32) Kopsa, H., Czech, W., Schmidt, P., Zazgornik, J., Pils, P. and Balcke, P. (1979) Use of thermography in kidney transplantation: Two year follow up study in 75 cases. *Proc. Eur. Dial. Transplant Assoc.* 16, 383-7.
33) Loughin, C.A. and Marino, D.J. (2007) Evaluation of thermographic imaging of the limbs of healthy dogs. *Am J Vet Res.* 68, 1064-9.
34) Lunstra, D.D. and Coulter, G.H. (1997) Relationship Between Scrotal Infrared Temperature Patterns and Natural-Mating Fertility in Beef Bulls. *J. Anim. Sci.* 75, 767–774.
35) Marr, C., McMillan, L., Boyd, J., Wright N.G. and Murray, M. (1993) Ultra sonographic and histopathological findings in equine superficial digital flexor tendon injury. *Equine Vet. J.* 25, 23-29.
36) Merla, A., Di Donato, L. and Romani, G.L. (2001) Assistance to surgery by means of infrared functional imaging: preliminary results. *Proceedings of 23rd Annual International Conference IEEE Eng. Med. Biol.* (Istanbul).
37) Merla, A. and Romani, G.L. (2006) Functional Infrared Imaging in Medicine: A Quantitative Diagnostic Approach, Engineering in Medicine and Biology Society. *Proceedings of EMBS '06. 28th Annual International Conference of the IEEE.*
38) Milano, C., Rocco, P., Paolino, G., Greco, P. and Lotti, G. (1982) Telethermographic aspect of the dorsolumbar region in idiopatic scoliosis. *Ital. J. Orthop.Traumatol* 8, 361-367.
39) Milazzo, M., Ludwig, N., and Redaelli, V. (2002) Evaluation of evaporation flux in building materials by infrared thermography. *6th International Conference on Quantitative Infrared Thermography, Dubrovnik, September 24-27.*

40) Ming, Z., Zaproudina, N., Siivola, J., Nousiainen, U. and Pietikainen, S. (2005) Sympathetic pathology evidenced by hand thermal anomalies in carpal tunnel syndrome. *Pathophysiology* 12, 137-41.
41) Misiolek, M., Namylowski, G. and Czecior, E. (1999) Thermography in the investigation of head and neck tumors. *Med Sci Monit* 5, 1187-1190.
42) Mitchell, W.S., Winocour, P.H., Gush, R.J., Taylor, L.J., Baker, R.D., Anderson, D.C. and Jayson, M.I. (1989) Skin blood flow and limited joint mobility in insulin-dependent diabetes mellitus.» *Br. J. Rheumatol.* 28, 195-200.
43) Moretti, J.L., Rigo, F., Bischof-Delaloy, A., Taillefer, R., Caillat-Vigneron, N. and Karcher, G. (1993) Medicina Nucleare in Diagnostica Clinica. Ed. It. A cura di Tarolo GL. Elsevier Masson Ed..
44) Nikkhah, A., Plaizier, J,C., Einarson, M.S., Berry, R.J., Scott, S.L. and Kennedy, A.D. (2005) Short communication: Infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. *Journal of Dairy Science* 88, 2749-2753.
45) Nyirjesy I., (1982) Breast thermography. *Clin Obstet Gynecol.* 25, 401-8.
46) Penninck, D. and D'Anjou, M-A. (2008) Atlas of Small Animal Ultrasonography. *Blackwell Publishing*.
47) Pozzi Mucelli, R. (1996) Trattato italiano di tomografia computerizzata. *Idelson*, vol.1.
48) Rich, P.B., Dulabon, G.R., Douillet, C.D., Liswa, T.M., Robinson, W.P., Zarzaur, B.L., Pearlstein, L. and Katz, L.M. (2004) Infrared thermography: a rapid, portable, and accurate technique to detect experimental pneumothorax. *J Surg Res* 120, 163- 70.
49) Ring E.F.J (2004) The historical development of thermal imaging in medicine *Rheumatology* 43 6): 800-802.
50) Robicsek, F., Masters, T.N., Svenson, R.H., Daniel, W.G., Daugherty, H.K., Cook, J.W. and Selle, J.G. (1978) The application of thermography in the study of coronary blood flow. *Surgery* 84, 858-6.
51) Schaefer, A.L., Jones, S.D.M, Murray, A.C., Sather, A.P. and Tong, A.K.W. (1989) Infrared thermography of pigs with known genotypes for stress susceptibility in relation to pork quality. *Can. J. Anim. Sci.,* 69, 491-495.
52) Schaefer, A.L., Cook, N.J., Tessaro, S.V., Deregt, D., Desroches, G., Dubeski, P.L., Tong, A.K.W. and Godson, D.L. (2003) Early detection and prediction of infection using infrared thermography. *Canadian Journal of Animal Science* 84, 73-80.
53) Schaefer, A.L., Perry, B.J., Cook, N.J., Miller, C., Church, J., Tong, A.K.W. and Stenzler, A. (2006) Infrared detection and nitric oxide treatment of bovine respiratory disease. *Online Journal of Veterinary Research Volume* 10, 7- 16.
54) Schwarz, T. and Saunders, J. (2011) Veterinary Computed Tomography. *Wiley-Blackwell.*
55) Schweinitz, D.G. (1999) Thermographic diagnostics in equine back pain. *Vet Clin North Am Equine Pract.* 15, 161–177.
56) Scott, S.L., Schaefer, A.L., Tong, A.K.W. and Lacasse, P. (2000) Use of infrared thermography for early detection of mastitis in dairy cows. *Canadian Journal of Animal Science* 80, 764-765.
57) Shah, A., Rao, K.H., Ruedi, B. and Magrini, G. (1984) Determination of fertility interval with ovulation time estimation using differential skin surface temperature (DST) measurment. *Fert. Steril* 41, 771-774.
58) Shamaa, A.A. and Gohar, H.M. (2002) Clinical Assessment of Thermography as a Diagnostic and Prognostic Tool in Horse Practice. *http://www.flirthermography.com/media/12Embay2002s.pdf.*
59) Sherman, R.A., Ernst, J.L. and Markowski, J. (1987) Differences between trunk heat patterns shown by complete and incomplete spinal cord injured veterans. *Paraplegia* 25, 466-474.

60) Skliarenko, R.T. and Zakalinskii, I.A. (1986) Role of thermography in the evaluation of the result of the surgical treatment of patient with arteriosclerosis obliterans. *Vestn. Khir.* 136, 49-53.
61) Smaga, Paszkowski, Wozniak, Walczak. (2003) Analysis of physiologic and abnormal pictures of uterine cervix by means of infrared thermography. *Ginekol Pol.* 74, 847-54.
62) So, Y.T., Aminoff, M.J. and Olney, R.K. (1990) A comparison of thermography and electromyography in the diagnosis of cervical radiculopathy. *Muscle nerve* 3, 1032-6.
63) So, Y.T., Aminoff, M.J. and Olney, R.K. (1989) Evaluation of thermography in the diagnosis of selected entrapment neuropathies. Neurology, 39, 1154-8.
64) Spence, V.A. and Walzer, W.F. (1984) The relationship between temperature isotherms and skin blood flow in the ischemic limb. *J. Surg. Res.* 36, 278-281.
65) Stashak, T. (1987) Adam's lameness in horses. *Fourth Ed., Lea and Febiger*, 100-156.
66) Stess, R.M., Sisney, P.C., Moss, K.M., Graf, P.M., Louie, K.S., Gooding, G.A. and Grunfeld, C. (1986) Use of liquid crystal thermography in the evaluation of the diabetic foot. *Diabetes Care* 9, 267-272.
67) Sun, P.C., Jao, S.H. and Cheng, C.K. (2005) Assessing foot temperature using infrared thermography. *Foot Ankle Int.* 26, 847-53.
68) Svartberg, K. (2005) A comparison of behaviour in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. *Appl. Anim. Behav. Sci.* 91, 103–128.
69) Tapernoux, B. and , Hessler, C. (1977) Thermography of malignant melanomas. *J Dermatol Surg Oncol.* 299-302.
70) Thrall, D.E. (2012) Textbook of veterinary diagnostic radiology. Elsevier, 6th Ed.
71) Tucker, A.T. (2000) Infrared thermographic assessment of the human scrotum. *Fertil steril* 74, 802-803.
72) Turner, T.A., Fessler, J.F., Lamp, M., Pearce, J.A. and Geddes, L.A. (1983) Thermographic evaluation of horses with podotrochlosis. *Am J Vet Res.* 44, 535-9.
73) Turner, T.A. (1991) Thermography as an aid to the clinical lameness evaluation. *Vet Clin North Am Equine Pract.* 7, 311-38.
74) Turner, T.A. (1996) Alternative methods of soft tissue imaging. *Dubai Int Equine Symp*, 165–176.
75) Weil, M., Litzke, L.F. and Fritsch, R. (1998) Diagnostic validity of thermography of lameness in horses. *Tierarztl Prax Ausg G Grosstiere Nutztiere* 26, 346-54.
76) Westbrook, C., Kaut Roth, C. and Talbot, J. (2011) MRI in Practice. *Wiley-Blackwell*, 4th edition.
77) Wright T., McGechan A. (2003) Breast cancer: new technologies for risk assessment and diagnosis. *Mol Diagn* 7, 49-55.
78) Yang, W-J., Zhang, N. and Andersen, H.F. (1990) Abdominal wall temperature variation during labor and parturition by infrared thermography. *1st World Congress of Biomechanics*.

Figure 1: Labrador retriever, male, 3 years old; medio-lateral view of elbow joint: severe degenerative joint diseases due to elbow osteoarthritis. Note the subchondral sclerosis of ulna and extensive periosteal reaction. Diagnosis: elbow dysplasia for fragmented coronoid process of the ulna (FCP).

Figure 2: Dobermann, male, 5 years old; medio-lateral view of stifle joint: moth-eaten osteolysis of distal femur and proximal tibia is present. Note the cortical erosion and interrupted periosteal reaction at caudal surface of femur and amorphous periosteal reaction at the level of caudal aspect of proximal tibia. Diagnosis: histiocytic sarcoma.

Figure 3: same dog of fig.2; CT slice obtained at the level of the distal femur: note the depth of bone disruption in femur condyles, not recognizable on survey radiographs (bone window).

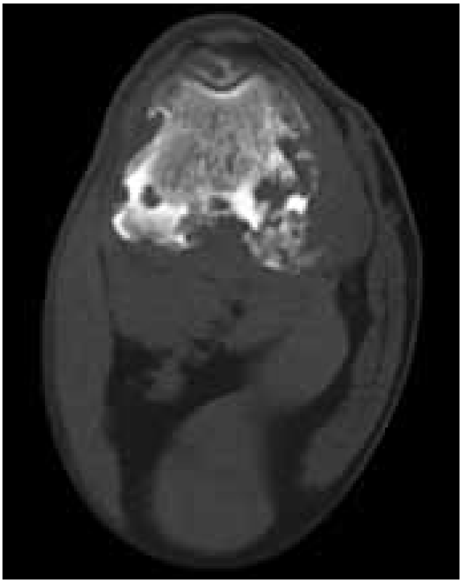


Figure 4: Corgie, neutered female, 6 years old; CT slice obtained at the level of the frontal sinuses: an extensive destruction of frontal bones is evident; a mixed periosteal reaction, amorphous on right frontal bone and sunburst on the left is also present (bone window). Diagnosis: Carcinoma of frontal sinuses.

Figure 5: DSH, neutered male, 7 years old; CT slice obtained at the level of the shoulders: a large round mass arising from subcutaneous soft tissues of the shoulder is well recognizable. This slice was obtained after administration of iodinated contrast medium: note the ring effect enhancement due to a large hypodense center consistent of necrotic tissue or hemorrhage (soft tissue window). Diagnosis: feline injection-site sarcoma.

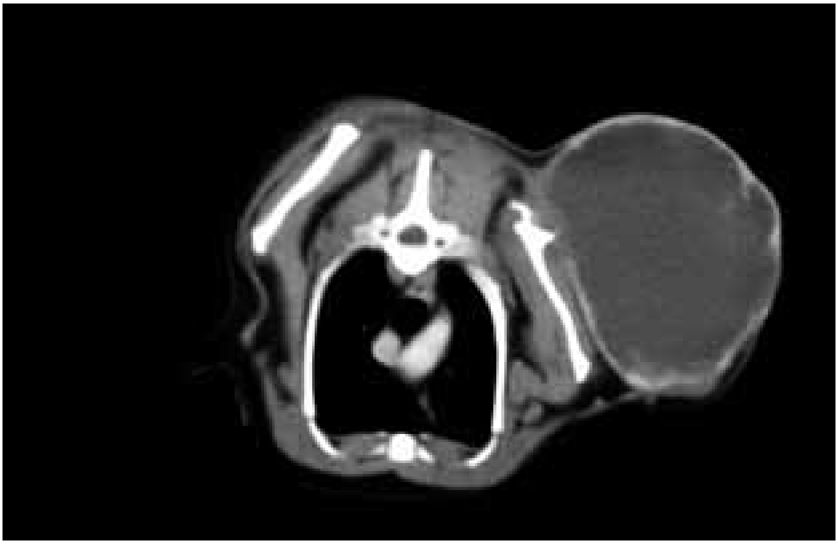


Figure 6: Boxer, neutered female, 10 years old; CT slice obtained at the level of thyroid after administration of iodinated contrast medium: an high and heterogeneous enhancement of both thyroid lobes is present. Note the sharp edge of the masses; no filling defect of vessels is recognizable (soft tissue window). Diagnosis: thyroid adenocarcinoma.

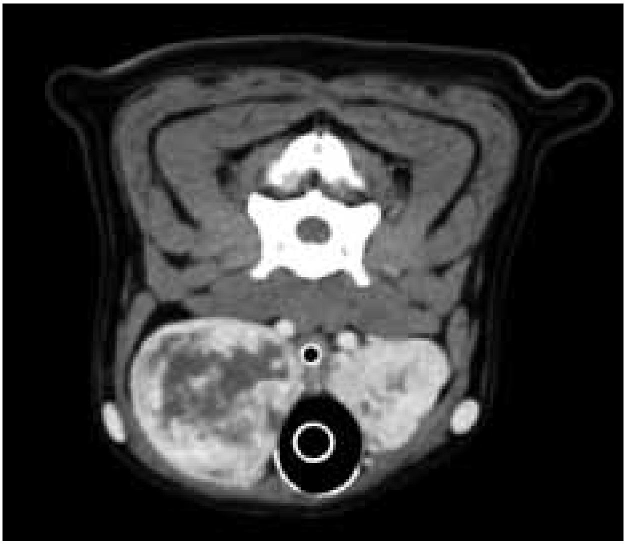


Figure 7: Mongrel dog, male, 5 years old; MRI sagittal view of lumbosacral spine T2-weighted: a moderate bulging of the spinal cord due to intervertebral disc compression is recognizable. Degeneration of the disc is responsible of the complete hypointensity of the signal in the intervertebral space (compare to L6-L7). Diagnosis: Hansen type II disc protrusion.

Figure 8: DSH, neutered male, 11 years old: MRI dorsal view of the skull at the level of the posterior orbital fossa Gradient echo weighted: an extensive soft tissue mass provided with poor margin is recognizable in the left posterior orbital fossa. Diagnosis: lymphoma.

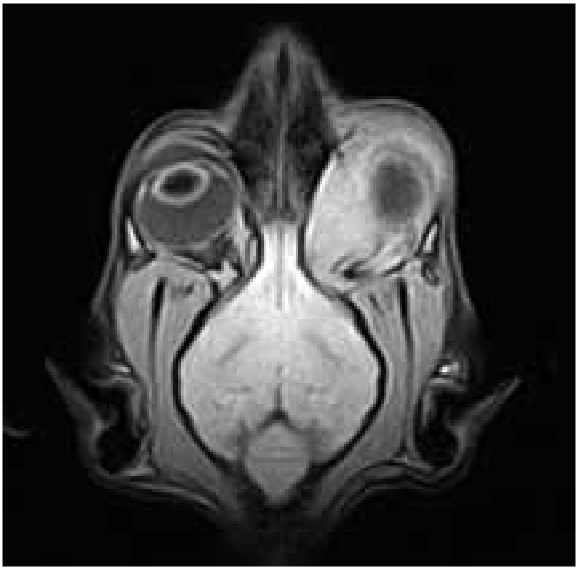


Figure 9: Thermography of a cow: inflammation of the right forelimb is evident in green-yellow.

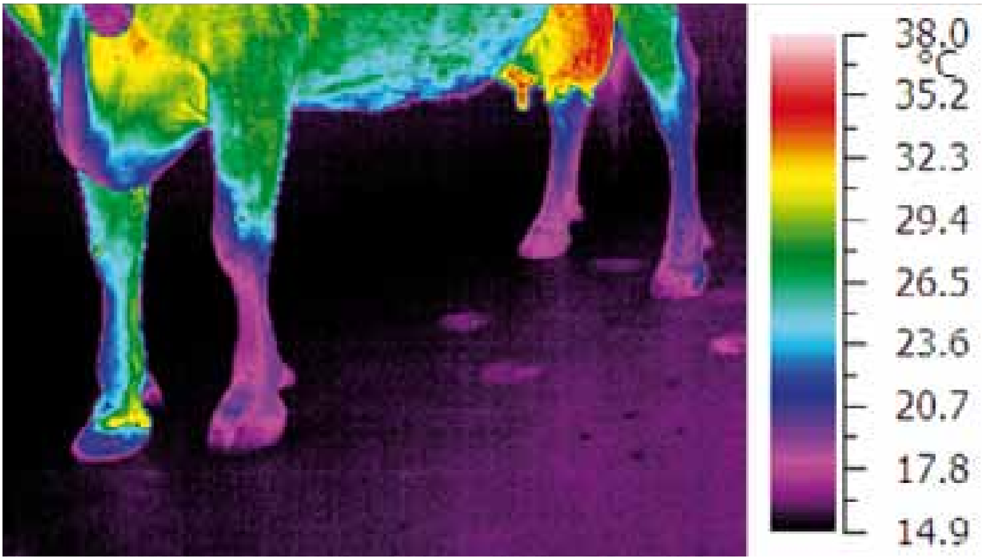


Figure 10: Thermographic images of a horse during treadmill testing.

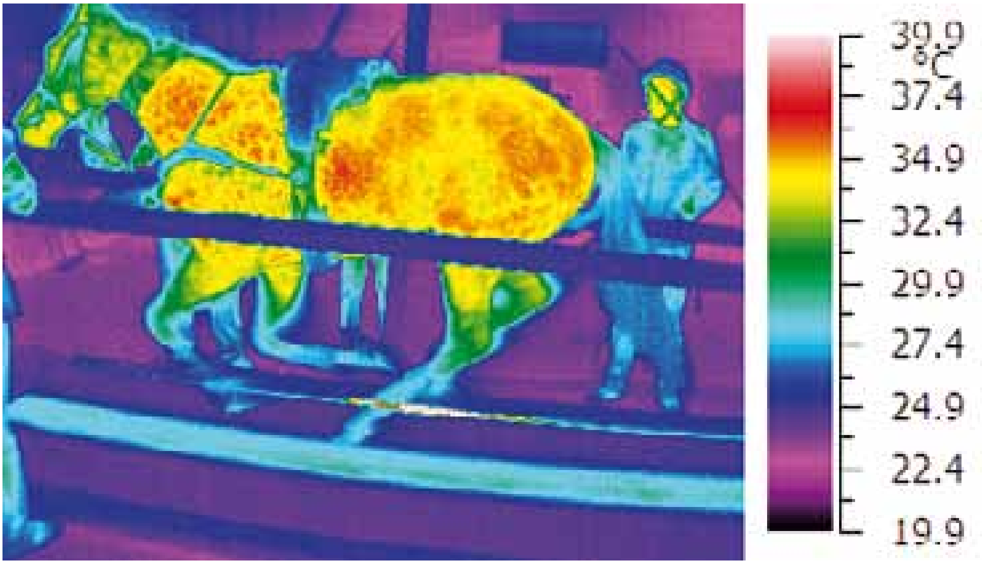
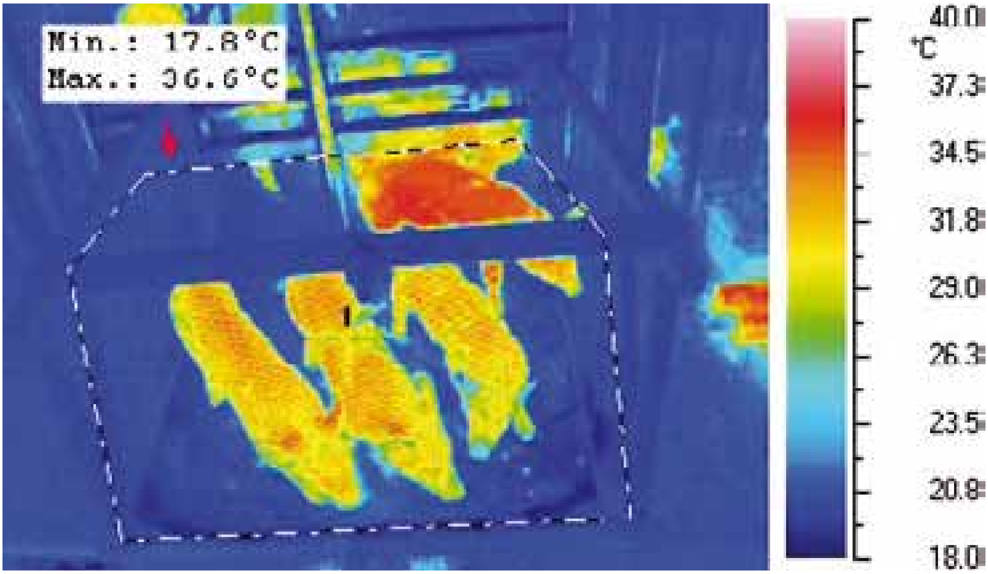
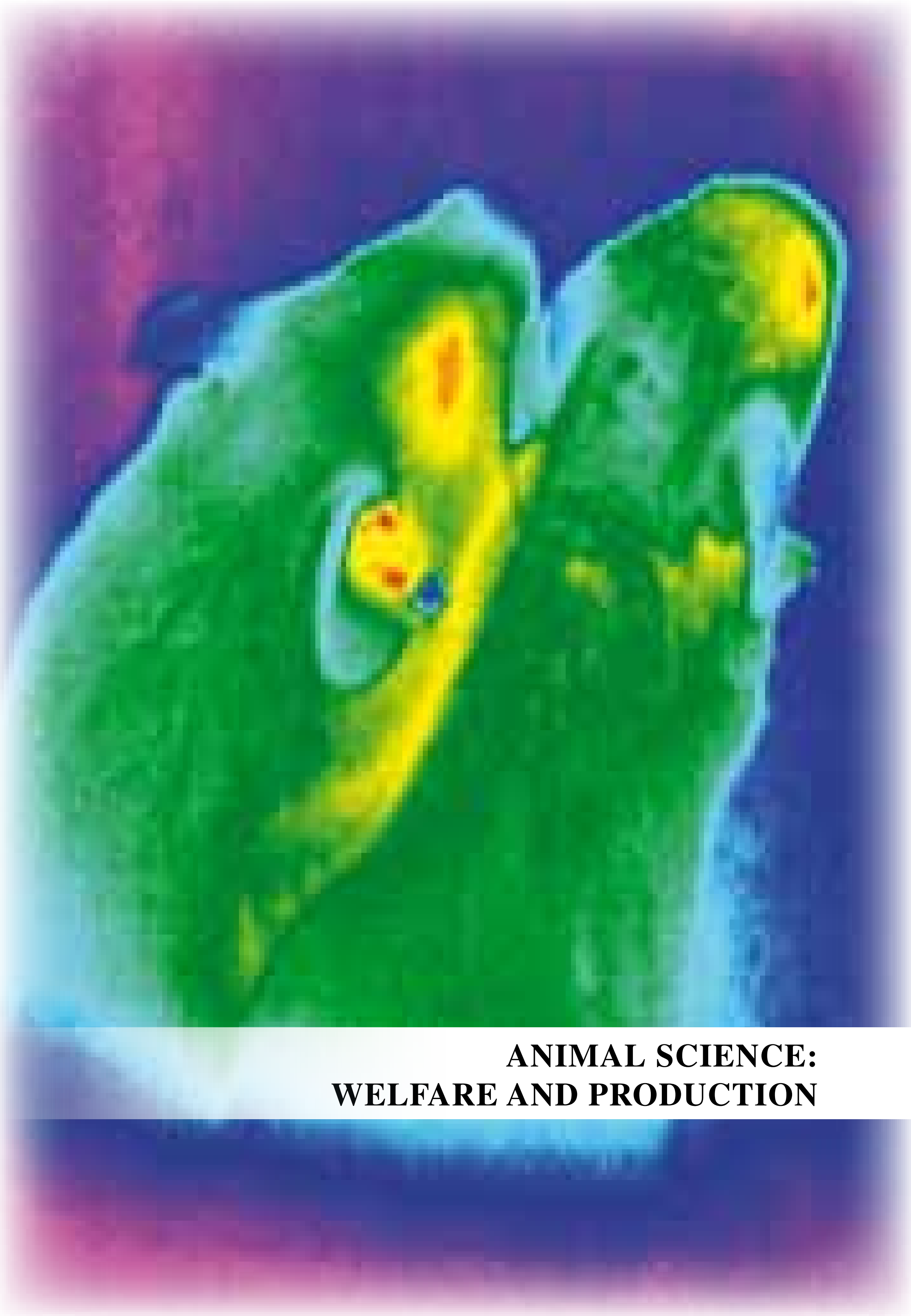


Figure 11:Thermographic image of piglets during transportation.





ANIMAL SCIENCE: WELFARE AND PRODUCTION

THERMAL IMAGING: THERMOREGULATION IN RELATION TO ANIMAL PRODUCTION AND WELFARE

MALCOLM A. MITCHELL

SRUC, the Roslin Institute Building, Easter Bush, Midlothian, EH25 9RG, UK

ABSTRACT

There are many potential applications of infra-red thermography or thermal imaging in the area of animal welfare and production. These include the measurement of body temperature and characterisation of thermal exchange in a range of challenging environments and during procedures such as handling, transport and slaughter. The effects of the imposition of various stressors and the welfare outcomes may be assessed by IRT. Thermal status of animals can be determined by IRT at important stages of development and during different production phases. The prediction of heat and cold stress and the definition of thermal comfort zones for each species and age of animals are objectives of research in animal welfare that can be underpinned by thermographic analysis. Thermography is also extremely useful in the detection and diagnosis of disease affecting animal welfare and in the examination of injuries, inflammation, lameness, muscle damage and fatigue and in pathological states altering metabolic rate or metabolic heat production in specific tissues or body regions. IRT can be used to monitor the physiological and welfare outcomes of a large number of routine procedures to which livestock are exposed. Thermography also allows the monitoring the efficacy of treatments and practices aimed at improving animal welfare and health and can be employed to monitor recovery from pathological states and injury and to assess healing. Thermography can also be used to characterise the affective or emotional state of animals in response to stressful, harmful or painful stimuli or procedures and thus to quantify the welfare outcomes.

Key words

Infrared thermography, animal welfare, body temperature, stress.

INTRODUCTION

Thermography and thermal imaging have a wide range of potential applications in studies relating to animal welfare and animal production. In addition to representing a useful methodology to complement other approaches in the assessment of welfare status in relation to thermoregulatory function, behaviour, stress and disease in experimental studies, measurements of surface temperatures might constitute a useful non-invasive procedure that might readily be applied in a commercial setting for purposes of welfare monitoring. The potential areas of application of thermography in terms of animal welfare may be usefully grouped in to 4 functional categories:

1 Thermoregulatory status and capacity in relation to environmental conditions or challenges (heat stress and cold stress) – changes associated with thermoregulatory behaviours;
2 Changes in body temperature and/or regional surface temperature in response to injury, disease, infection, pathology and inflammatory responses;

3 Consequences of exposure to stressors inducing changes in cardiovascular responses, vasomotor tone and local perfusion and heat exchange e.g. mutilations, surgical or identification procedures – stress and pain responses – autonomic responses;
4 Responses in body temperature and local temperatures associated with other procedures to which animals re exposed e.g. handling, transportation.

The advantages of non-invasive imaging techniques have been promoted in relation to biomedical research (Hudson 2005). Imaging methodologies allow animals to be monitored in a temporal and spatial manner that facilitates acquisition of greater volumes of data on smaller numbers of animals. In addition imaging allows study of functional details in a live animal with minimal restraint or interference (Hudson 2005). Thermography or thermal imaging shares these advantages with other forms of imaging. The application of infrared thermography in research on animals has been reviewed by McCafferty (2007) who has emphasised all the practical advantages relating to the approach. It is non-invasive and studies may be designed to be minimally intrusive, thus, having little influence upon the animals' normal behaviours or activities and imposing little or no stress upon the subjects. The use of infrared thermography (IRT) as a non-invasive tool to study animal welfare has been reviewed by Stewart et al (2005). It is clear that many studies of animal welfare involve the assessment and quantification of stress in terms of the magnitude of stressors imposed upon the animal and the measurement of the physiological and behavioural responses of the animal to that stressor. As Stewart et al (2005) emphasise, often, the measurement of stress responses as animal outcomes may involve the imposition of further stress. It is proposed that infra-red thermography constitutes a reliable and effective method to assess both acute and chronic stress in animals. It is suggested that appropriate application of IRT can allow measurement of different components of the stress axis including acute sympathetic and hypothalamic-pituitary-adrenocortical responses. Those authors have continued to apply productively, IRT to a number of important animal welfare concerns including measurement of stress in dairy cows (Stewart et al 2007), detection of fear related responses in cattle during handling (Stewart et al 2008a), responses to disbudding in calves (Stewart et al 2008b) and autonomic responses of cattle to other painful procedures (Stewart et al 2010a and b). These studies illustrate that thermography may be utilised to address many aspects of animal welfare through an understanding of the physiological mechanisms of stress and behavioural responses to stressors and selection of the appropriate thermal measurements.

APPLICATIONS OF THERMOGRAPHY TO ANIMAL WELFARE AND PRODUCTION

Thermoregulatory status and capacity in relation to environmental conditions or challenges (heat stress and cold stress) – changes associated with thermoregulatory behaviours

In the course of production animals may be exposed to thermal micro-environments that may impose demands upon the thermoregulatory system resulting in heat stress or cold stress. Regulation of the core body temperature will require responses in the behavioural and physiological mechanisms controlling heat production and heat loss (Mitchell 2013 – this publication). Thermography may be employed to monitor body temperature responses and thus the degree of heat stress or cold stress experienced and the welfare risks imposed by the challenge or to examine and quantify heat exchange at the animals' surface and to characterise the thermoregulatory responses exhibited. There are essentially two ways in which thermal imaging may be employed in the examination of thermal stress in relation to animal welfare and production.

Firstly, data relating to the surface temperatures of livestock species under various thermal conditions may be employed in the development of models of thermal exchange and thermoregulatory requirements and the outputs employed to predict thermal balance and the effects and consequences of quantified thermal challenges in experimental or commercial conditions. Such models have been developed for a number of different species (Turnpenny et al 2000 a and b; Fiahlo et al 2004; Huynh et al 2005) including specifically ratites (Phillips and Sanborn 1994), horses (Morgan et al 1997; Autio et al 2006, 2007; Valle et al 2011), barn owls (McCafferty et al 1998), pigs (Loughmillar et al 2000; Huynh et al 2005), cattle (Keren and Olson 2006), turkeys (Yahav et al 2011), and penguins and seals (McCafferty et al 2011 and Paterson et al 2012). Such models may also be used to predict the functional activity and significance of "thermal windows" in the thermoregulatory responses of livestock and other animal species to defined thermal loads (e.g. vultures (Ward et al 2008), elephants (Weissenbock et al 2010), camels (Abdoun et al 2012) and harbour seals (Nienaber et al 2010; Erdsack et al 2012.). The degree of cold stress experienced by cattle during outdoor wintering has been modelled using surface temperature measurements and predicted heat loss and altered energy requirements (Keren and Olson 2007), a technique than can be extended to other species to assess the welfare risks associated with such husbandry procedures. Prediction of the degree of heat or cold stress experienced by animals in the face of a thermal challenge allows assessment of the welfare consequences of the exposure and may allow definition of acceptable range and limits of thermal loads required to optimise animal welfare in practice (Mitchell and Kettlewell 1998).

Secondly, thermography may be used to monitor the effects of thermal challenges upon body temperature and heat exchange regulation animals as a direct assessment of the consequences for homeostatic success and effort and therefore, for animal welfare. In cows exposed to cold and wet conditions in New Zealand IRT was employed in conjunction with many other physiological measures to provide integrated assessment of the stress imposed and the physiological and behavioural adaptations to cold and therefore the reduction in welfare (Webster et al 2008). Thermal imaging has provided insight in to the thermoregulatory capacity and physiological responses of South American Camelids and the roles of thermal windows and coat structure in the face of the thermal challenge (Schwalm et al 2006: Gerken 2010). Estimation of imposed heat load and heat dissipation efficiency has been correlated with other stress measures including serum cortisol profiles in studies on horses using thermography (Dupre et al 2010). IRT has been employed also to evaluate the thermal stress imposed in exercising horses during exposure to high temperature (de Moura et al 2011). The effects of "thermal windows", both natural and artificial, in the feather cover of chickens upon heat exchange during heat stress have been examined using IRT (Gerken et al 2006). These studies support the concept of developing strategies to increased heat loss from commercial poultry during heat stress tin order to improve welfare and productivity. Heat loss for a potential thermal window i.e. the foot of the turkey is illustrated in Figure 1 (SRUC 2013 ©). Assessment of thermal comfort is an important aspect of animal welfare. The physiological and behavioural responses of cattle to a cycling diurnal variation in thermal loads have been characterised by (Zahner et al 2004) and the relationship between THI (temperature humidity index) and surface temperature measured by IRT has been described. Thus optimum temperature ranges for each species or age of animal can be derived from appropriate studies of thermal balance and identification of critical temperatures e.g. thermographic evaluation of lower critical temperature in weanling horses (Autio et al 2007). Thermoregulation in new born piglets is a matter of some welfare concern as in large litter piglets failing to feed (reach a teat) will rapidly succumb to hypothermia. Redaelli et al (2011a) have employed IRT video to fully characterise the thermal relations of piglets immediately after farrowing and to examine the efficacy of heat floors and bedding in the prevention of hypothermia. The piglets' skin temperature fell from 39.6°C at birth to 31-32°C within 10 minutes and only returned to 35°C one hour later if the piglet quickly reached the udder and fed. Figure 2 shows piglets sucking soon after birth. Thermal comfort indices have been related to thermographic temperatures in lambs (Paim et al 2012). That study identified the most appropriate sites to measure surface temperature in these animals to assess thermal comfort and reported that these thermographic temperatures were good indicators of environmental and thermal comfort conditions. In addition to modelling studies determining thermal comfort based upon environmental temperature alone (e.g. Do Nascimento et al 2011) other work has addressed the role of additional environmental factors such as air movement. Thus IRT may be employed to characterise the effects of air movement upon heat exchange and thermoregulation an approach that has bee applied to both poultry (Yahav et al 2004; Yahav and Giloh 2012) and pigs (Geers et al 1987). Recently, regional temperature changes measured by IRT have been compared in different genetic lines of lambs in response to heat and cold challenge to identify any differences in thermal tolerance (Paim et al 2013).

Changes in body temperature and/or regional surface temperature in response to injury, disease, infection, pathology and inflammatory responses

Many regard disease and injury as the biggest threat to animal welfare in modern production systems. Both infectious diseases and metabolic pathology may result in patho-physiological changes that will influence, heat production and loss mechanisms and that will influence local blood flow and heat exchange and thus surface temperatures. Other chapters of this publication deal with some aspects of these phenomena in more detail.

Schaefer et al (2004) propose that IRT can be used in the development of an early prediction index for infection in calves. Schaefer et al (2012) also indicate that IRT might be employed in the North American cattle industry for the large scale detection and monitoring of bovine respiratory disease (BRD) which constitutes a major health and welfare concern. The febrile state following administration of bacterial endotoxin (lipopolysaccharide – LPS) to dairy cattle can be readily detected by IRT (Willard et al 2007). Hovinen et al (2008) recommended the use of thermal imaging in commercial dairies for the early detection of elevated udder skin temperatures in clinical mastitis. Polat et al (2010) have proposed that IRT represents a useful method for detection of elevated “udder skin surface temperature” in mastitis in dairy cows but questioned the applicability in different lines and environmental conditions. Pezeshki et al (2011) suggest that IRT does not provide a useful early detection of mastitis through elevated udder temperature in cows infected with *E. coli*. Poikalainen et al (2012) have presented evidence that in routine practice IRT maybe effectively employed in the detection and diagnosis of mastitis, leg injuries body surface damage and problems with milking hygiene in a commercial setting and may constitute an essential part of any future precision cattle farming system.

Haley et al (2005) suggested that infrared thermography might provide early detection of lameness in cattle. Nikkhah et al (2005) have proposed that thermography used to measure foot temperatures in cows during early lactation would be a useful method to monitor hoof health. This contention is supported by the more recent reports of Stokes et al (2012) and Alsaaod and Buscher (2012) who confirmed the relationships between hoof surface temperatures, the presence of hoof lesions and lameness. The use of IRT in the diagnosis and control and treatment of hoof lesions in cattle represents an important contribution to animal welfare. In horses thermography is regarded as an important tool in the diagnosis and management of lameness and has been employed to identify laminitis, sole abscess, generalised hoof inflammation and back injuries (Eddy et al, 2001; van Hoogmoed and Snyder 2002). IRT may also bee utilised to detect non-legitimate administration of analgesic and neurolytic agents in sports horses. Figure 3 shows a thermogram of the hind feet of a cow affected by lameness (SRUC 2013 ©). In figure 4 a steer with suspected lameness exhibits a “laminitic stance” and requires further detailed analysis of the possible pathology. Figure 5 shows a turkey with lameness and inflammation of the left hock joint (SRUC 2013 ©))

Dunbar et al (2009) have advocated thermography as a rapid, remote and reliable method for the preliminary detection of foot and mouth disease (FMD) in Mule Deer and propose that the approach be used to screen animals in order to make decisions relating to further diagnostic tests and treatments, however, in cattle Gloster et al (2011), suggest that IRT of the hoof is, at best, a modest predictor of early FMD.

IRT may be usefully employed to calculate mean body surface temperature in pigs infected with *acintobacter* and to detect the febrile response over a wide range of environmental temperatures (Loughmiller et al 2001). It has been proposed (Wilcox et al 2009) that IRT provides a useful tool for the screening of "bumblefoot" (Staphylococcus aureus infection) in poultry with a correlation of 83% between initial IRT images and diagnosis and subsequent (14 days later) visual scoring ($p<0.001$).

Consequences of exposure to stressors inducing changes in cardiovascular responses, vasomotor tone and local perfusion and heat exchange, e.g. mutilations, surgical or identification procedures – stress and pain responses – autonomic responses

There are a number of stimuli that may induce alterations in peripheral vascular tone and local blood flow. These changes mediated by the autonomic nervous system (ANS) direct environmental stressors or psychological stressors. Thus, IRT has been employed to characterise changes in cutaneous and body temperatures during and after conditioned "fear to context" in rats (Vianna and Carrive 2005). It was demonstrated that fear and arousal induced a strong vasoconstriction in specific body areas that could be readily measured by IRT. This contextual conditioned fear in rats induces a hyperthermia of non-shivering origin which IRT indicates does not involve the inter-scapular brown adipose tissue deposits (Marks et al 2009). In rabbits the periocular area, the eye surface and the ear are areas that exhibit vasomotor response to stress that may be assessed using IRT (Ludwig et al 2007). In poultry, mild stressors such a loud noise and restraint are associated with vasomotor responses in a range of surface locations but with the most pronounced response in the comb (Edgar et al 2009). It was concluded that thermal imaging is a viable sensitive indicator of mild distress in chickens. IRT may also be used to assess the autonomic response associated with ostensibly positive experiences. Thus, in laying hens the anticipation and consumption of a signalled palatable reward are accompanied by a decrease in peripheral temperature as measured by IRT of the comb (Moe et al 2012). The finding was interpreted as reflecting a peripheral vasoconstriction more indicative of emotional arousal rather than emotional valence.

In rhesus monkeys (*Macaca mulattta*) IRT revels a decrease in nasal temperature associated with a negative emotional state induced by the approach of a "threatening human" (Nakayama et al 2005) and in further trial by "monkey emotions as manifested by conspecific emotional behaviours and expressions" using video images and digitised sound (Kuraoka and Nakamura 2011). The authors propose that nasal temperature constitutes a useful indicator of animals' emotional state. The stress experienced by horses in response to involvement in competition may be assessed by IRT to determine changes in eye temperature (Valera et al 2012) and the technique is sensitive to the phases of stress development and the findings correlate well with estimates of the concomitant cortisol response. The application of devices such as double bridles or "jaw-clamping crank nosebands" to horses, induce stress and an associated ANS responses indicated by increased eye temperature monitored by IRT (McGreevy et al 2012).

The ANS and peripheral temperature responses to more severe stressors are useful indicators of the severity of the treatment particularly when coupled to other stress indices. Thus, during painful stimuli such as surgical castration, in particular without anaesthesia, there is a marked increase in eye temperature and altered heart rate variability parameters (Stewart et al 2010 a and b). It was suggested that the findings of that study are indicative of a vasodilation in the eye which may attributable to the pain induced release of vasodilator agents or parasympathetic activation following the initial sympathetic response to the acute pain. The changes in heart rate variability support the latter explanation. The use of measurement of transient changes in eye temperature by IRT to assess acute stress is a useful approach and has been productively applied to the removal of velvet antlers in the Wapiti (Cook and Schaefer 2002) and the disbudding of calves (Stewart et al 2008; Coetzee 2011). The value of incorporating other indices such as heart rate variability in to the protocols for the assessment of stress in such procedures and like areas of welfare concern must be emphasised.

IRT may also be employed to asses the welfare issues associated with other procedures such as rumeno-centesis for the diagnosis of conditions such as sub-acute ruminal acidosis (Gianesella et al 2010) by monitoring of skin temperatures in the region of the region of the incision and following the rate of healing and recovery. Similarly IRT can be used to assess the presence and extent of sensitivity and pain following procedures such as tail docking (Eicher et al 2006) in conjunction with other physiological and behavioural indicators.

Responses in body temperature and local temperatures associated with other procedures to which animals re exposed e.g. handling, transportation

IRT monitoring of eye temperature has been employed to assess the degree of stress imposed by handling procedures in cattle (Stewart et al 2008a). In relation to animal transport, early studies (Gariepy et al 1989) suggested that IRT of pigs before stunning at the slaughterhouse could detect both PSE and DFD conditions. These meat quality problems are attributable to pre-slaughter stress and as such may indicate and reflect the welfare status of the animals in transit and perhaps even prior to the journey. Brown et al (2005) have examined the effects of loading and unloading pigs on to transport vehicles and include surface temperature measurement as an important non-invasive index of imposed stress. In a companion study the same authors correlated surface (ear) temperature with mean blood temperature at slaughter and concluded the surface measure was a valid index of changes in body temperature and the degree of stress experienced by the animals (Warriss et al 2006a). The possible welfare benefits of mechanical ventilation systems on commercial pig transport vehicles were investigated also by examining the thermoregulatory responses to three standard journeys on an experimental vehicle with both natural and mechanical ventilation regimes operational simultaneously of different decks (Warriss et al 2006b). It was claimed that ear temperature was a useful index of body temperature and the degree of thermal challenge experienced in transit and that the measure is a useful adjunct to other welfare assessment measures in animal transport studies. This view has been supported by Nanni-Costa (2008) and a more recent report details the application of IRT to assessment of stress and welfare during the road transportation of piglets (Nanni-Costa et al 2011). Physiological stress modelling has been established as a basis for improvements in welfare of animals during commercial transportation through the use of animal outcomes to define thermal comfort zones in transit (Mitchell and Kettlewell1998, 2008; Mitchell 2005; Villarroel et al 2011). A fundamental component of these models is the assessment of deep body temperature and thermal exchange and as such thermography represents an important approach. The use of surface temperature measurements in these models has been reviewed (Mitchell 2013).Figures 6 and 7 show thermograms of sheep during and after road transportation (SRUC 2013 ©).

Differential IRT measurement of surface temperatures of the flank and rump of cattle has been reported to be a valid indicator of methane production by reflecting ruminal activity, metabolism and heat production (Montanholi et al 2008). A similar principle and approach has been adopted for the detection and diagnosis of Sub-acute ruminal acidosis (SARA) using IRT to detect differences in flank temperature in affected cattle (Gatto et al 2010). As SARA represents an extremely important issue in relation to both animal welfare and productivity in the dairy and beef industries the provision of a non-invasive detection and monitoring system will represent a major advance.

OTHER APPLICATIONS OF IRT IN RELATION TO ANIMAL WELFARE

Van den Heuvel et al (2004) have described the use of IRT to examine peripheral heat loss in relation to sleep patterns in humans. Currently there is interest in sleep patterns in livestock, particularly intensively produced species, in relation to stress and welfare. It is suggested that application of IRT in these studies may prove beneficial.

Schwartzkopf-Genswein et al (1997) have examined the welfare consequences of different branding methods in cattle (hot iron and freeze branding) and have assessed the degree of thermal insult, inflammation and tissue damage associated with each method by IRT analysis. The technique is particularly useful for characterisation of tissue injury and local inflammation in such studies and may find application in similar procedures such as tail docking, ear clipping and tooth clipping and grinding. Redaelli et al (2011b) have examined the temperatures reached in the teeth and surrounding tissue during the grinding procedure using IRT video to assess the welfare implications of the procedure. Average temperature during 2 seconds grinding was 50°C with a peak temperature of 88°C. There was limited heating of surrounding soft tissue.

In studies of nociception in cattle carbon dioxide lasers have been employed to cause controlled thermal stimulation of skin nociceptors in the skin over the metatarsi of calves (Veissier et al 2000). The latency and magnitude of leg withdrawal in the animals were used as the behavioural indicators of induced pain. The temperature of the skin target (38-78°C) and the thus the stimulus strength were monitored by means of IRT.

In wild sea birds pollution is a serious problem in relation to soling of feather cover and breakdown of waterproofing and insulation. This constitutes a major animal welfare issue. The degree of damage to the feathers and the efficacy of waterproofing may be determined is affected birds by IRT (Anon 2012). The recovery of the birds during rehabilitation may also be monitored by surface temperature analysis of the feathers in wetted birds.

SUMMARY

There are many potential applications of infra-red thermography or thermal imaging in the area of animal welfare and production. These include the measurement of body temperature and characterisation of thermal exchange in a range of challenging environments and during procedures such as handling, transport and slaughter. The effects of the imposition of various stressors and the welfare outcomes may be assessed by IRT. Thermal status of animals can be determined by IRT at important stages of development and during different production phases. The prediction of heat and cold stress and the definition of thermal comfort zones for each species and age of animals are objectives of research in animal welfare that can be underpinned by thermographic analysis. Thermography is also extremely useful in the detection and diagnosis of disease affecting animal welfare and in the examination of injuries, inflammation, lameness, muscle damage and fatigue and in pathological states altering metabolic rate or metabolic heat production in specific tissues or body regions. IRT can be used to monitor the physiological and welfare outcomes of a large number of routine procedures to which livestock are exposed. Thermography also allows the monitoring the efficacy of treatments and practices aimed at improving animal welfare and health and can be employed to monitor recovery from pathological states and injury and to assess healing. Thermography can also be used to characterise the affective or emotional state of animals in response to stressful, harmful or painful stimuli or procedures and thus to quantify the welfare outcomes.

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REFERENCES

1) Abdoun, K.A., Samara, E.M. Okab, A.B. and Al-Haidary A.A. (2012) Regional and circadian variations of sweating and body surface temperature in camels (Camelus domedarius). Animal Science Journal, 83, 556-561.
2) Alsaaod, M., and Buscher, W. (2012) Detection of hoof lesions using digital infrared thermography in dairy cows. J. Dairy Sci. 95 (2), 735-742.
3) Anon (2012) Seabird thermography project: California Department of Fish and Wildlife. http://www.dfa.ca.gov/ospr/Science/marine-wildlife-vetcare/SeabirdThermography.
4) Autio, E., Heiskanen, M-L., and Mononen, J. (2007) Thermographic evaluation of the lower critical temperature in weanling horses. Journal of Applied Welfare Science, 10 (3), 207-216.
5) Autio, E. Neste, R., Airaksinen, S and Heiskanen, M-L. (2006) Measuring the heat loss in horses in different seasons by infrared thermography. Journal of Applied Animal Welfare Science, 9:211-221.
6) Brown, S.N., Knowles, T.G., Wilkins, L.J., Chadd, S.A and Warriss, P.D. (2005) The response of pigs to being loaded or unloaded onto commercial animal transporters using three systems. Veterinary Journal 170 (1), 91-100.
7) Coetzee, J.F. (2011) A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Applied Animal Behaviour Science 135 (3), 192-213.
8) Cook, N.J., and Schaefer, A.L. (2002) Stress responses of wapiti (*Cervus elaphus canadensis*) to removal of velvet antler. Canadian Journal of Animal Science 82 (1), 11-17.
9) Dunbar, M.R., Johnson, S.R., Ryan, J.C., and McCollum, M. (2009) Use of infrared thermography to detect thermographic changes in mule deer (*Odocoileus hemionus*) experimentally infected with foot-and-mouth disease. Journal of Zoo and Wildlife medicine 40 (2), 296-301.
10) Dupre, Y., Strohm, A., Keis, E., Harney, J., Moulton, K., and Ryan, P.L. (2010) assessing heat load and dissipation using digital thermography and serum cortisol profiles in horses during the summer months. Journal of Dairy Science 93, 761-761.
11) Eddy, A.L., van Hoogmoed, L.M., and Snyder, J.R. (2001) The role of thermography in the management of equine lameness. The Veterinary Journal 162 (3), 172-181.
12) Edgar, J.L., Paul, E.S., and Nicol, C.J. (2009) Thermal imaging as a non-invasive tool to assess mild distress in chickens. Poultry Welfare Symposium, Cerva, Italy, May 18-22nd 2009.
13) Eicher, S.D., Cheng, H.W., Sorrells, A.D., and Schutz, M.M. (2006) *Short Communication:* Behavioural and physiological indicators of sensitivity or chronic pain following tail docking. Journal of Dairy Science 89 (8), 3047-3051.

14) Erdsack, N., Hanke, F.D., Denhardt, G. and Hanke, W. (2012) Control and amountof heat dissipation through thermal windows in the harbour seals (Phoca vitulina). Journal of Thermal Biology, 37:537-544.
15) Fialho, F.B., Bucklin, R.A., Zazueta, F.S. and Myers, R.O. 2004. Theoretical model of heat balance in pigs. Animal Science, 79, 121-134.
16) Gariepy, C., Amiot, J. and Nadai, S. (1989) Ante-mortem detection of PSE and DFD by infrared thermography of pigs before stunning. Meat Science 25 (1), 37-41.
17) Gatto, M., Gianesella, M. and Morgante, M. (2010) Application of infrared thermography as tools to diagnosis subacute ruminal acidosis of dairy cows. Large Animal Review 16, 7-12.
18) Geers, R., van der Hel, W., Verhagen, J., Verstegen, M., Goedseels, V., Brandsma, H., Hencken, A., Scholler, J., and Berckmans, D. (1987) Surface temperatures of growing pigs in relation to the duration of acclimation to air temperature or draught. Journal of Thermal Biology 12 94), 249-255.
19) Gerken, M. (2010) Relationships between integumental characteristics and thermoregulation in South American camelids. Animal 4 (9), 1451-1459.
20) Gerken, M., Afnan, R., and Dorl, J. (2006) adaptive behaviour in chickens in relation to thermoregulation. Archiv. Fur. Gerflugelkunde 70 (5), 199-207.
21) Gianesella, M., Morgante, M., Stelletta, C., Ravarotto, L., Giudice, E and van Saun, R.J. (2010) Evaluating the effects of rumenocentesis on health and performance in dairy cows. ACTA VET. BRNO 79, 459-468.
22) Gloster, J., Ebert, K., Gubbins, S., Bashiruddin, J., and Paton, D.J. (2001) Normal variation in thermal radiated temperature in cattle: implications for foot-and-mouth disease detection. BMC Veterinary Research 7:73.
23) Haley, D.B., Bench, C.J., de Passille, A.M., Rushen, J., Lepage, P., Coplyn, J., and Schaefer, A.L. (2005) The use of infrared and exercise to non-invasively determine lameness in dairy cattle. Journal of dairy Science 88 (Suppl. 1), 134-134.
24) Hovinen, M., Siivonen, J., Taponen, S., Hanninen, L., Pastell, M., Aisla, A-M., and Pyorala, S. (2008) Detection of clinical mastitis with help of a thermal camera. Journal of Dairy Science 91 (12), 4592-4598.
25) Hudson, M. (2005) The welfare and scientific advantages of non-invasive imaging of animals used in biomedical research. Animal Welfare 14, 303-317.
26) Huynh, T.T.T., Aarnink, A.J.A, Verstegen, M.W.A., Gerrits, W.J.J., Heetkamp, M.J.W, Kemp, B. and Canh, T.T. (2005) Effects of increasing temperatures on physiological changes in pigs at different relative humidities. Journal of Animal Science 83 (6), 1385-1396.
27) Keren, E.N. and Olson, B.E. (2007) Applying thermal imaging software to cattle grazing winter range. Journal of Thermal Biology 32, 204-211.
28) Kuraoka, K., and Nakamura., K. (2011) The use of nasal skin temperature measurements in studying emotion in macaque monkeys. Physiology and Behaviour 102 (3-4), 347-355.
29) Ludwig, N., Gargano, M., Luzi, F., Carenzi, C., and Verga, M. (2007) Technical Note: Applicability of infrared thermography as non-invasive measurement of stress in rabbit. World Rabbit Sci. 15, 199-206.
30) Loughmiller, J.A., Spire, M.F., Dritz, S.S., Fenwick, B.W., Hosni, M.H., and Hogge, S.B. (2001) Relationship between mean body surface temperature measured by use of infrared thermography and ambient temperature in clinically normal pigs and pigs inoculated with *Actinobacillus pleuropneumoniae*. American Journal of Veterinary Research 62 (5), 676-681.
31) Marks, A., Vianna, D.M.L., and Carrive, P. (2009) Nonshivering thermogenesis without interscapular brown adipose tissue involvement during conditioned fear in the rat. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 296 (4), 1239-1247.

32) McCafferty, D.J. (2007) The value of infrared thermography for research on mammals: previous applications and future directions. Mammal review 37 (3), 207-223.
33) McGreevy, P., Warren-Smith, A., and Guisard, Y. (2012) The effects of double bridles and jaw-clamping crank nosebands on temperature of eyes and facial skin of horses. Journal of Veterinary Behaviour 7, 142-148.
34) Mitchell, M.A. (2005) Using physiological models to define environmental control strategies. Editors R. Gous and T. Morris, CAB International, Oxon, UK, pp 209-228.
35) Mitchell, M.A and Kettlewell, P.J. (2008) Engineering and design of vehicles for long distance road transport of livestock (ruminants, pigs and poultry). Veterinaria Italiana 44(1) 213-225.
36) Moe, R.O., Stubsjoen, S.M., Bohlin, J., Flo, A and Bakken, M. (2012) Peripheral temperature drop in response to anticipation and consumption of a signalled palatable reward in laying hens (*Gallus domesticus*). Physiology and Behaviour 106, 527-533.
37) Montanholi, Y.R., Odongo, N.E., Swanson, K.C., Schenkel, F.S., McBride, B.W. and Miller, S.P. (2008) Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (*Bos Taurus*). Journal of Thermal Biology 33 (8), 468-475.
38) De Moura, D.J., Maia, A.P.D., Vercellino, R.D., Medeiros, B.B.L., Sarubbi, J., and Griska, P.R. (2011) Infrared thermography to evaluate the training horse thermoregulation. Engenharia Agricola 31 (1), 23-32.
39) Nakayama, K., Goto, S., Kuraoka, K., and Nakamura, K. (2005) Decrease in nasal temparature of Rhesus monkeys (*Macaca mulatta*) in negative emotional state. Physiology and Behaviour 84, 783-790.
40) Nanni-Costa, L. (2009) Short-term stress: the case of transport and slaughter. Ital. J. Anim. Sci, 9 (Suppl. 1) 241-252.
41) Nanni-Costa, L., Redaelli, V., Magnani, D., Cafazzo, S., Amadori, M., Razzuoli, E., Verga, M. and Luzi, F. (2011) Relationship between piglets skin temperature measured by infrared thermography and environmental temperature in a vehicle in transit: a preliminary study. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 112, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
42) Do Nascimento, G.R., Pereira, D.F., Naeaes, I de A., and Rodrigues, L.H.A (2011) Thermal comfort fuzzy index for broiler chickens. Engenharia Agricola 31 92), 219-229.
43) Nienaber, J., Thonton, J., Horning, M., Polasek, L. and Mellish, J-A. (2010) Surface temperature patterns in seals and sea lions: A validation of temporal and spatial consistency. Journal of Thermal Biology, 35:435-440.
44) Nikkhah, A., Plaizier, J.C., Einarson, M.S., Berry, R.J., Scott, S.L., and Kennedy, A.D. (2005) Short communication: Infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. Journal of Dairy Science 88 (8), 2749-2753.
45) Paim, T.P., Borges, B.O., Lima, P.M.T., Dallago, B.S.L., Louvandini, H., and McManus, C. (2012) Relation between thermographic temperatures of lambs and thermal comfort indices. International Journal of Applied Animal Sciences 1 (4), 108-115.
46) Paim, T.P., Borges, B.O., Lima, P.M.T., Gomes, E.F., Dallago, B.S.L., Fadel, R., de Menezes, A.M., Louvandini, H., canozzi, M.E.A., Barcellos, J.O.J. and McManus, C. (2013) thermographic evaluation of climatic conditions on lambs from different genetic groups. International Journal of Biometeorology. 57 91), 59-66.
47) Paterson, W., Sparling, C.E., Thompson, D., Pomeroy, P.P., Currie, J.I. and McCafferty, D. (2012) Seals like it hot:Changes in surface tempertuare of harbour seals (Phoca vitulina) from late pregnancy to moult. Journal of Thermal Biology, 37:454-461.

48) Pezeshki, A., Stordeur, P., Wallemacq, H., Schynts, F., Stevens, M., Boutet, P., Peelman, L.J., de Spiegeleer, B., Duchateau, L., Bureau, F., and Burvenich. (2011) Variation of inflammatory dynamics and mediators in primiparous cows after intramammary chellenge with *Escherichia coli*. Veterinary Research 42:15.
49) Poikalainen, V., Praks, J., Veermae, I., and Kokin, E. (2012) Infrared temperature patterns of cow's body as an indicator for health control at precision cattle farming. Agronomy Research: Biosystem Engineering Special issue 1, 187-194.
50) Phillips, P.K. and Sanborn, A. (1994) An infra-red, thermographic study of surface temperature in three ratites: ostrich, emu and double-wattled cassowary. Journal of Thermal Biology, 19:423-430.
51) Polat, B., Colak, A., Cengiz, M., Yanmaz, E., Oral, H., Bastan, A., Kaya, S., and Hayirli, A. (2010) Sensitivity and specificity of infrared thermography in detection and subclinical mastitis in dairy cows. Journal of Dairy Science 93, 3525-3532.
52) Redaelli, V., Farish, M., Luzi, F. and Baxter, E. (2011a) Demonstration of thermoregulatory control of piglets during farrowing by infrared thermography. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 88, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
53) Redaelli, V., Luzi, F. Verga, M. and Farish, M. (2011b) Thermographic techniques to assess welfare during teeth grinding in piglets. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 87, Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
54) Schaefer, A.L., Cook, N., Bench, C., Chabot, J.B., Colyn, J., Liu, T., Okine, E.K., Stewart, M., and Webster, J.R. (2012) The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. Research in veterinary Science 93 92), 928-935.
55) Schaefer, A.L., Cook, N., Tessaro, S.V., Deregt, D., Desroches, G., Dubeski, P.L., Tong, A.K.W., and Godson, D.L. (2004) Early detection and prediction of infection using infrared thermography. Canadian Journal of Animal Science 84 (1), 73-80.
56) Schwalm, A., Erhardt, G., Gerken, M., and Gauly, M. (2006) The influence of high ambient temperature on thermoregulation, thyroid hormone and testosterone levels in male llamas (*Lama glama*) depending in their fibre length. In (eds) gerken, M., and Renueri, C. South American Camelids Research Vol 1, proceedings, pp115-120.
57) Schwartzkopf-Genswein, K.S. and Stookey, J.M. (1997) The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle. Canadian Journal of Animal Science 77(4) 577-583.
58) Stewart, M., Schaefer, A.L., Haley, D.B., Colyn, J., Cook, N.J., Stafford, K.J., and Webster, J.R. (2008a) Infrared thermography as a non-invasive method for detecting fear-related responses of cattle to handling procedures. (2008a) Animal Welfare 17 (4), 387-393.
59) Stewart, M., Stafford, K.J., Dowling, S.K., Schaefer, A.L., and Webster, J.R. (2008b) Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiology and Behaviour 93, 789-797.
60) Stewart, M., Verkerk, G.A., Stafford, K.J. Schaefer, A.L., and Webster, J.R. (2010a) Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. J. Dairy Sci. 93 (8), 3602-3609.

61) Stewart, M., Webster, J.R., Schaefer, A.L., Cook, N.J., and Scott, S.L. (2005) Infrared thermography as a non-invasive tool to study animal welfare. Animal Welfare 14, 319-325.
62) Stewart, M., Webster, J.R., Stafford, K.J. Schaefer, A.L., and Verkerk, G.A. (2010b) *Technical note:* Effects of an epinephrine infusion on eye temperature and heart rate variability in bull calves. J. Dairy Sci. 93 (11), 5252-5257.
63) Stewart, M., Webster, J.R., Verkerk, G.A., Schaefer, A.L., Colyn, J., Cook, N.J., and Stafford, K.J.(2007) Non-invasive measurement of stress in dairy cows using infrared thermography. Physiology and Behaviour 92, 520-525.
64) Stokes, J.E., Leach, K.A., main, D.C.J., and Whay, H.R. (2012) An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foor lesions in dairy cattle. The Veterinary Journal 193 (3), 674-678.
65) Turnpenny, J.R., McArthur, A.J., Clark, J.A., Wathes, C.M., 2000. Thermal balance of livestock. 1. A parsimonious model. Agr. Forest Meteorol. 101, 15-27.
66) Turnpenny, J.R., Wathes, C.M., Clark, J.A., McArthur, A.J., 2000. Thermal balance of livestock. 2. Applications of a parsimonious model. Agr. Forest Meteorol. 101, 29-52.
67) Valera, M., Bartolome, E., Sanchez, M.J., Molina, A., Cook., N., and Schaefer, A.L. (2012) Changes in Eye temperature and stress assessment in horses during show jumping competitions. Journal of Equine Veterinary Science 32, (12), 827-830.
68) Valle, E, Redaelli, V., Papa, M., Bergero, D and Luzi, F. (2011) Infrared thermography: a non-invasive technique to assess metabolic activity in horses. Proceedings of the 5th International Conference on the Assessment of Animal Welfare at Farm and Group Level, WAFL 2011, page 113 Campbell Centre for the Study of Animal Welfare, University of Guelph, Guelph, Ontario, Canada, August 8th-11th 2011, Eds, T. Widowski, P. Lawlis and K. Sheppard, Wageningen Academic Publishers, the Netherlands (DOI: 10.3921/978-90-8686-738-7.
69) Van den Heuvel, C.J., Ferguson, S.A., Gilbert, S.S. and Dawson, D. (2004) Thermoregulation in normal sleep and insomnia: the role of peripheral heat loss and new applications for digital thermal infrared imaging (DITI). Journal of Thermal Biology 29(7-8) 457-461.
70) Van Hoogmoed, L.M., and Snyder, J.R. (2002) Use of infrared thermography to detect injections and palmar digital neurectomy in horses.
71) Veissier, I., Rushen, J., Colwell, D. and de Passille, A.M. (2000) A laser-based method for measuring thermal nociception of cattle. Applied Animal Behaviour Science 66, (4) 289-304.
72) Vianna, D.M.L and Carrive, P. (2005) Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. European Journal of Neuroscience 21 (9), 2505-2512.
73) Villarroel, M., Barreiro, P., Kettlewell, P., Farish, M. and Mitchell, M.A. 2011. Time derivatives in air temperature and enthalpy as non-invasive welfare indicators during long distance animal transport. Biosystems Engineering, 110 (3), 253-260.
74) Ward, J., McCafferty, D.J., Houston, D.C. and Ruxton, G.D. (2008) Why do vultures have bald heads? The role of postural adjustment and bare skin areas in thermoregulation. Journal of Thermal Biology, 33:168-173.
75) Warriss, P.D., Brown, S.N., Knowles, T.G., Wilkins, L.J., Pope, S.J., Chadd, S.A., Kettlewell, P.J. and Green, N.R. (2006b) Comparison of the effects of fan-assisted and natural ventilation of vehicles on the welfare of pigs being transported to slaughter. Veterinary Record 158, 585-588.
76) Warriss, P.D., Pope, S.J., Brown, S.N., Wilkins, L.J., and Knowles, T.G., (2006a) Estimating the body temperature of groups of pigs by thermal imaging. Veterinary Record 158 (10), 331-334.

77) Webster, J.R., Stewart, M., Rogers, A.R., and Verkerk, G.A. (2008). Assessment of welfare from physiological and behavioural responses of New Zealand dairy cows exposed to cold wet conditions. Animal Welfare 17 (1), 19-26.
78) Weissenbock, N.M., Weiss, C.M., Schwammer, H.M. and Kratochvil, H. (2010) Thermal windows on the body surface of African elephants (Loxodonta Africana) studied by infrared thermography. Journal of Thermal Biology, 35:182-188.
79) Wilcox, C.S., Patterson, J., and Cheng, H.W. (2009) use of thermography to screen for subclinical bumblefoot in poultry. Poultry Science 88, 1176-1180.
80) Willard, S., Dray, S., Farrar, R., McGee, M., Bowers, S., Chromiak, A., and Jones, M. (2007) Use of infrared thermal imaging to quantify dynamic changes in body temperature following lipopolysaccharide (LPS) administration in dairy cattle. Journal of Animal Science 85 (Suppl 2) 26-26.
81) Yahav, S., Straschnow, A., Luger, D., Shinder, D., Tanny, J., and Cohen, S. (2004) ventilation, sensible heat loss, broiler energy, and water balance under harsh environmental conditions. Poultry Science 83 (2), 253-258.
82) Yahav, S., Druyan, S., Rusal, M. and Shinder, D. (2011) Diurnally cycling temperature and ventilation affect young turkeys' performance and sensible heat loss. Journal of Thermal Biology, 36: 334-339.
83) Yahav,S and Giloh, M. (2012). Infrared Thermography - Applications in Poultry Biological Research, Infrared Thermography, Dr. Raghu V Prakash (Ed.), ISBN: 978-953-51-0242-7, InTech, Available from: http://www.intechopen.com/books/infrared-thermography/infrared-thermography-applications-in-agricultureand- biological-research.
84) Zahner, M., Schrader, L., Hauser, R., Keck, M., Langhans, W., and Wechsler, B. (2004) The influence of climate conditions on physiological and behavioural parameters in dairy cows kept in open stables. Animal Science 78, 139-147.

Figure 1: Thermogram of a turkey foot under cold conditions indicating reduced blood flow to the toes and reduced heat loss from this potential "thermal window".

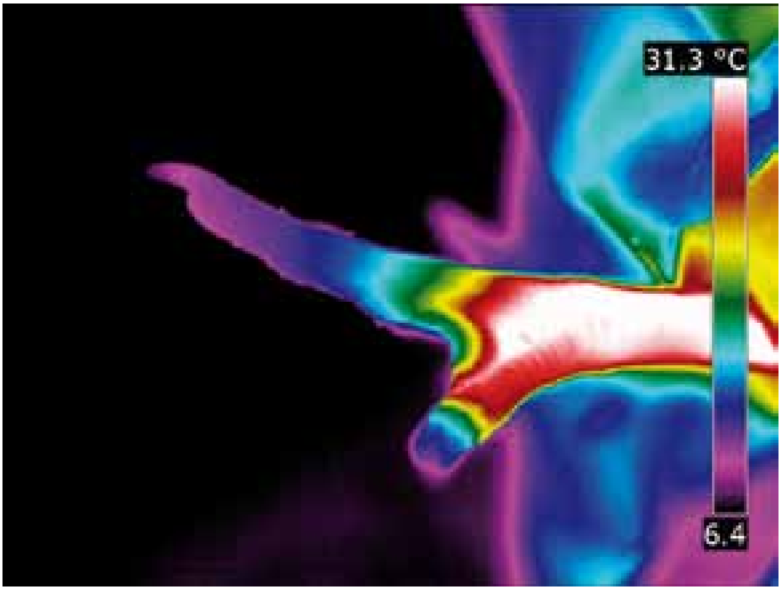


Figure 2: Thermogram of piglets sucking soon after birth.

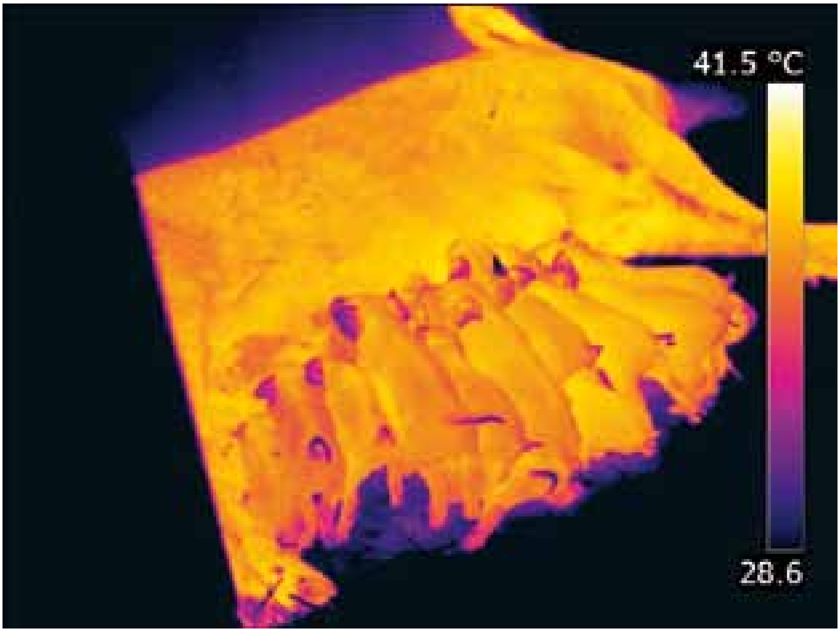


Figure 3: Thermogram of the hind feet of a cow walking. There is some indication of inflammation in the right foot and joints and the animal showed sign of lameness.

Figure 4: Thermogram of a steer in a "laminitic stance".

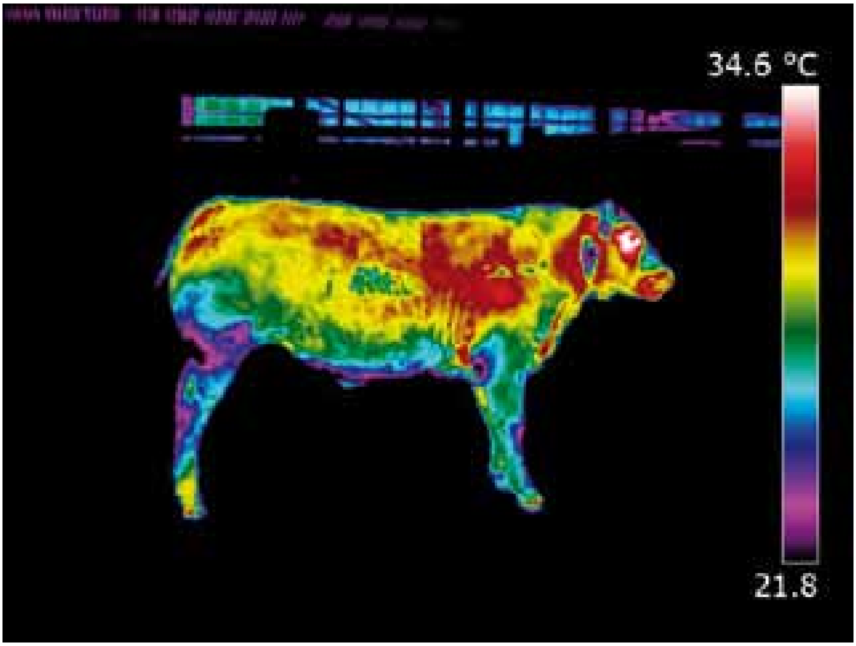


Figure 5: Thermogram of a turkey with lameness and inflammation of the left hock.

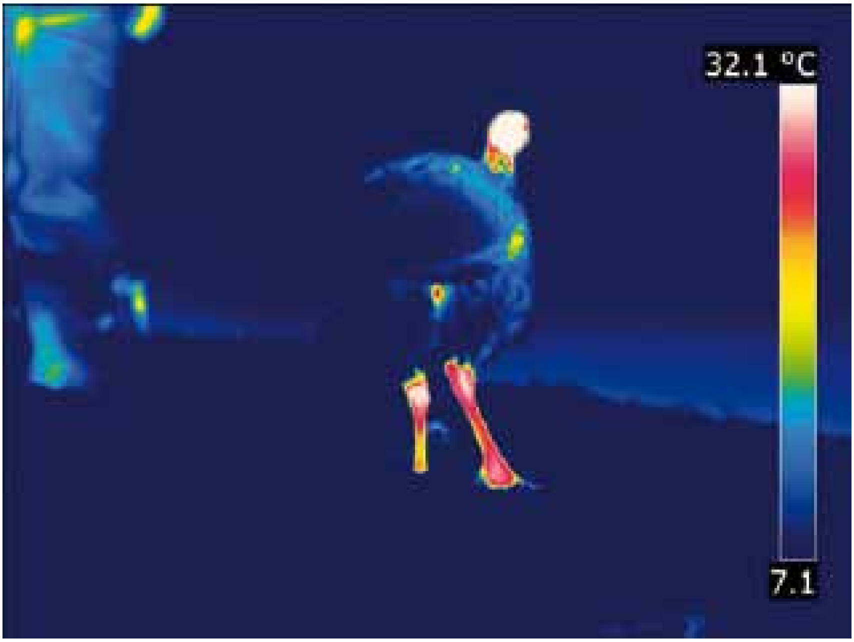


Figure 6: Thermogram of the head and face of a sheep on a transport vehicle after a journey.

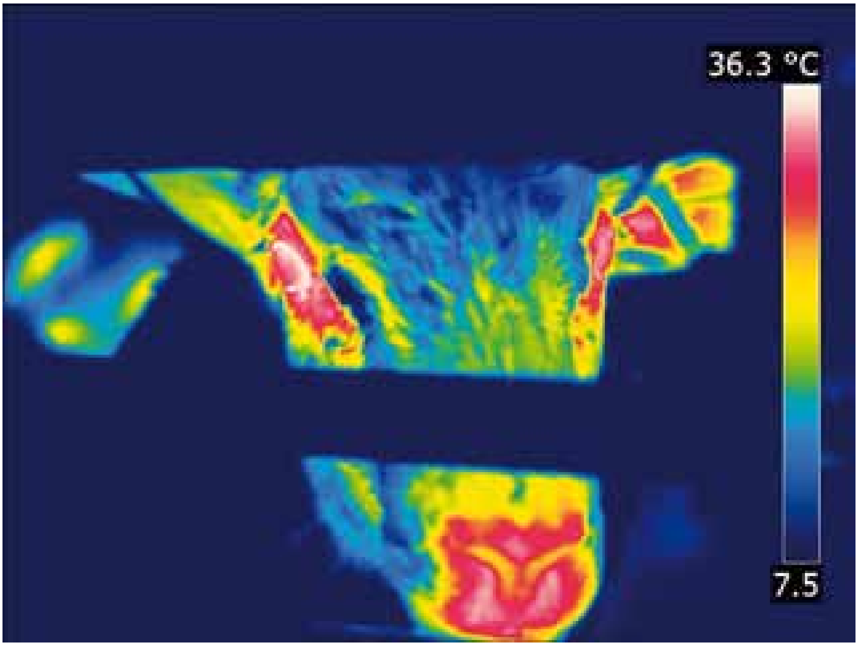
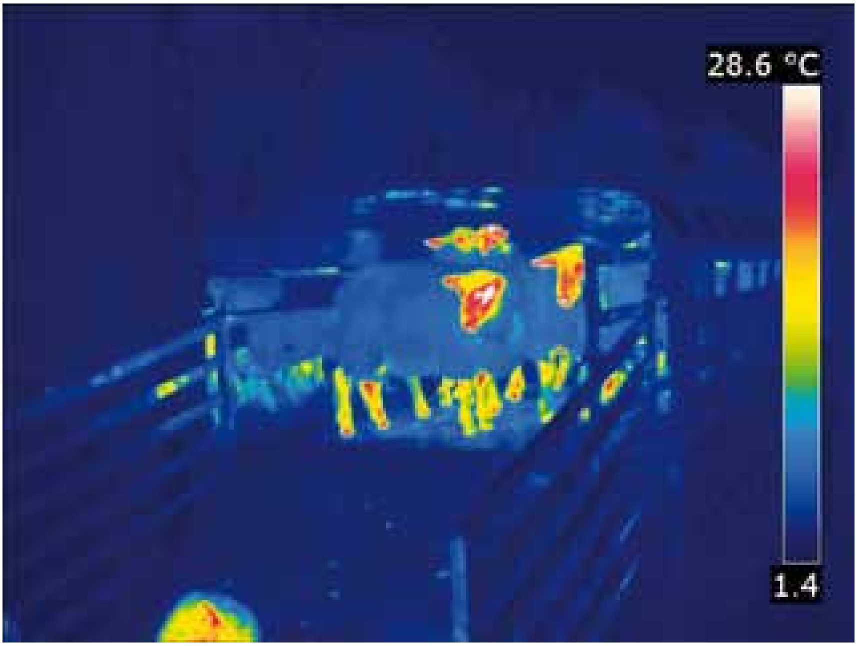


Figure 7: Thermogram of sheep being unloaded from a vehicle.



INFRARED THERMOGRAPHY IN LABORATORY ANIMALS

MASSENZIO FORNASIER

Siena Biotech, Siena, Italy

ABSTRACT

In the last years, the continuous improving of technologies has allowed to reduce the use of live animals in research and largely replace them with in vitro and in silico approaches. Nevertheless, the study of complex mechanisms such as cardiovascular function and thermoregulation, still need the in vivo methods and researchers are pushing for more and more refined methods, possibly non invasive, and as much as possible predictive of the clinical outcome in humans. The use of radiotelemetry and infrared thermography (IRT) allows for assessing multiple parameters useful not only for safety assessment of new chemical entities, biologics or medical devices but also to evaluate animal welfare in the different experimental and pre-experimental settings. These technologies allow also defining earlier experimental endpoints (humane endpoints) contributing to reduce the overall severity of procedure and identify and remove unwanted stressors in laboratory animals.

Key words

laboratory animals, animal welfare, radiotelemetry, infrared thermography, body temperature, blood pressure, heart rate, humane endpoints.

APPLICATION OF TELEMETRY AND THERMOGRAPHY IN LABORATORY ANIMALS

Background

In recent years, the continuous development of sophisticated technologies in the field of biomedical research has allowed for a progressive refinement of research models with greater emphasis on *in silico* and *in vitro* tools. Nevertheless, the use of *in vivo* models is still required to understand the complex interactions between organs and tissues in an intact body. The comprehension of the overall effect of physiological or pathological stimuli on the animal model gives the rational basis for testing the potential advantages of surgical or pharmacological intervention in clinical use. Due to the extreme complexity of these mechanisms, the effort of the research community is focused on developing experimental models in which data acquisition is accurate and reliable and, at the same time, the recording system does not interfere with the “normal” physiology and behaviour of the animal. Hence, the interest for remote monitoring systems, without disturbing effects due to interaction with the observer. This is particularly relevant for behavioural studies but also when monitoring cardiovascular or respiratory parameters or body temperature.

Telemetry systems: how they work

Radiotelemetry is measurement based on data transmitted by radio waves from the subject to the recording apparatus. Radiotelemetry is a well known technique used since the early 1950’s in several industrial fields and has a number of different applications, including motor racing, meteorology, flight control, etc. Radiotelemetry has been used in biology (biotelemetry) for vital parameters (mainly ECG) monitoring in emergency care units and for tracking purposes in wildlife monitoring (radio collars) (http://www.ericlwalters.org/ telemetry.pdf). The first implantable devices for lab animals were described in late 1980's (Clement, 1989; Brockway, 1991), and since the beginning, the advantage of a device placed inside the animal, with no risk of self-damage arising from other animals in the cage appeared evident. In the following years, more miniaturized devices also became available for mice, in which the major problem was the relatively unfavorable dimension of the transmitter compared to the animal body mass. The availability of more powerful batteries allowed for using implantable devices in larger experimental animal species (dogs and monkeys), in which the limiting factor was the power needed for transmitting the radio signal over a longer distance within the pen or the cage.

A telemetry setup is typically formed by the transmitter, one or more receivers placed outside the cage to collect the radio signals from the animal and a computerised acquisition system. The computer system acquires the data continuously, calculates derived parameters (peak values, duration, ratios etc.) and detects possible abnormal values or waveforms. Some types of transmitters are equipped with more than one sensor and this allows the recording of multiple signals (pressure, bio-potential and temperature) simultaneously from the same animal. Different transmitter models have dimensions suitable for the different animal species, from mice to monkeys. The main limitation of these devices is still the battery life and since there is no way to recharge the battery from the outside of body, devices need to be removed from the animal to be refurbished. To extend the lifespan of the battery, the electronics can be turned on and off from outside the body by means of magnetic switches.

Telemetry in preclinical research

Telemetry has been widely used in the last 20 years to understand physiology and pharmacology in many different animal models (Kramer, 2003), and for testing for any potentially adverse effects of new drugs on main vital functions (cardiovascular, respiratory and CNS) in preclinical species before the first trials in humans. The reliability and predictability to humans of telemetry studies in animals is acknowledged by the regulatory authorities and is considered as the method of choice (gold standard) for Safety Pharmacology studies (http://www.ich.org/products/guidelines/safety/article/safety-guidelines .html). It is worth noting that the large amount of data collected from pre-dose measures and observations and during the method setup have given a unique opportunity to record the "normal" status of animals and support the interpretation of changes related to the routine operations in the animal facility (caging, handling, dosing, restraint, etc.) (Duke 2001). These data have been used to "measure" the impact of stress factors on animal physiology and the ability of the animal to cope with such stressors. Furthermore, telemetry data were used to define the humane endpoints for animal experiments and evaluate the most suitable methods for animal euthanasia (Turner, 2012). Curiously, telemetry has also been used to monitor the effect of implantation surgery on animal physiology and welfare, leading to the consolidation of the best practice in this field (Morton, 2003).

Telemetry has been widely used in combination with other techniques, i.e. with video tracking in behavioural studies or with plethysmography in respiratory studies or in combination with calorimetry and infrared thermography in metabolism studies.

Telemetry and body temperature

Body temperature is commonly recorded in telemetry studies and physiological variations have been studied in different conditions such as sleep and arousal (Benstaali, 2001), physical exercise (Angle, 2011), pregnancy (Daneshvar, 2010). Telemetry body temperature changes have been recorded in experimental infections with different microbiological agents (Williamson 2007), or under different anesthesia protocols (Dispersyn, 2009). Body temperature changes were recorded to evaluate the effect of different caging systems (Kemppinen, 2010; Giral, 2011) or the effect of common experimental procedures such as handling and dosing (Sharp, 2003) or the effect of interaction with cage mates (Nicholson, 2009; Van Loo, 2007) and giving very helpful information to establish the best practices in animal colony management.

Telemetry and Thermography

Of particular interest is the combination of core body temperature measured with telemetry and skin temperature with infrared thermography (IRT). The combination of these two techniques were used to investigate the mechanism of thermoregulation, in particular during the perinatal period or during hibernation (Jonasson, 2012) and the role of non-shivering thermogenesis and brown adipose tissue (BAT) located in the inter-scapular region. Skin temperature recordings allowed the monitoring of the kinetics of thermal dispersion in the different species, i.e. in the ear (in rabbits) or in the tail (for rodents).

Interesting data have been generated with the combination of telemetry and IRT to understand the effect of psychological stress (conditioned fear) in the rat (Vianna, 2005). In this experimental model, the decrease of skin temperature in the tail and limb extremities (-5.3 and -7.5 °C, respectively) was accompanied by an increase of core temperature (1 °C) for the entire duration of the experiment (30 min). Once the fear stimuli were removed, an increase of tail skin temperature (+ 3.3 °C) was recorded.

Peripheral vasoconstriction is a well known mechanism connected to the "fight and flight" mechanism to reduce blood loss in case of injury, but interestingly the effect of fear stimuli was significantly higher in conditioned vs non-conditioned animals, suggesting that the psychological component of fear is relevant. In the same experimental model (Marks, 2009) showed that the body temperature changes were not linked to BAT activation, suggesting that the mechanisms of thermogenesis during fear stress are different from those observed in the hypothermic stress.

Combined telemetry and thermography measurement of body temperature gave interesting information to explain behaviour in neonates of altricial species (rabbits and rodents) and the strategy to prevent hypothermia in the first days after birth (Gilbert, 2012). In these species, the activation of BAT represents the main mechanism of thermogenesis but this would not be so effective without the innate behavior of pups known as "huddling". This allows the reduction of heat dispersion and allows the BAT cells to be maintained at a temperature at which they can work more effectively. Overall, huddling allows pups to use the metabolic energy coming from food for growth instead for thermal production, and leads to the conclusion that, within their litter, young altricial mammals compete for food but cooperate for warmth.

Body temperature as Humane Endpoint

A fall in body temperature has been proposed as an early experimental endpoint indicator to suggest the need for preemptive euthanasia to alleviate terminal distress and permit timely collection of biologic samples (Stokes, 2002).

Infrared thermography measurements have been proved to be reliable in comparison with rectal probes and telemetry in hypothermic mice via prolonged anesthesia or by injection of endotoxin (Newsom, 2004).

In experimental infections, hypothermia was the most valuable characteristic for distinguishing mice that would survive or succumb to the challenge, but the overall predictive ability of this measure varied substantially depending on the specific model (Trammel, 2011). Similar results were found in studies aimed to predict spontaneous death in aged inbred mice such as AKR/J mice, which die at a relatively young age due to the development of lymphoma, as well as male C57BL/6J and BALB/cByJ mice (Ray, 2010; Trammel, 2012)

Conclusions

Quantitative measurement and monitoring of physiological parameters over time are key elements to define the concept of "stress" in experimental protocols and evaluate the cost/benefit ratio of animal use in research. Telemetric techniques alone, or preferably in combination with other techniques such as IR thermography, represent a fundamental tool for the implementation of the "3Rs" (reduction, refinement, replacement) concept. The definition of the severity of the procedure, the choice of euthanasia method and the validation of humane endpoints, are only few examples of the benefit of this technique in defining the optimal experimental conditions and maximise the probability of obtaining scientifically valid results from animal studies.

REFERENCES

1) Angle T.C. and Gillette R.L. (2011). *Telemetric measurement of body core temperature in exercising unconditioned Labrador retrievers.* Can J Vet Res. Apr;75(2):157-9.
2) Benstaali C., Mailloux A., Bogdan A., Auzéby A. and Touitou Y. (2001) *Circadian rhythms of body temperature and motor activity in rodents their relationships with the light-dark cycle.* Life Sci. May 4;68(24):2645-56.
3) Brockway B.P., Mills P.A. and Azar S.H. (1991). *A new method for continuous chronic measurement and recording of blood pressure, heart rate, and activity in the rat via radiotelemetry*. Clin Exp Hypertens A 13:885–895.
4) Clement J.G., Mills P. and Brockway B. (1989) *Use of telemetry to record body temperature and activity in mice.* J Pharmacol Methods 21:129-140
5) Daneshvar H., Edwards T., Voss K., Landis K., Gleason T. and Atterson P. (2011). *Cardiovascular assessment in radiotelemetry-implanted pregnant rats.* J Invest Surg.; 24 (1): 35-43.
6) Dispersyn G., Pain L. and Touitou Y. (2009). *Circadian disruption of body core temperature and rest-activity rhythms after general (propofol) anesthesia in rats.* Anesthesiology. Jun; 110(6):1305-15.
7) Duke J.L., Zammit T.G. and Lawson D.M. (2001) *The effects of routine cage-changing on cardiovascular and behavioral parameters in male Sprague-Dawley rats*. Contemporary Topics in Laboratory Animal Science. 40(1): 17-20.
8) Gilbert C., McCafferty D.J., Giroud S., Ancel A. and Blanc S. (2012). *Private heat for public warmth: how huddling shapes individual thermogenic responses of rabbit pups.* PLoS One. 7(3):e33553.
9) Giral M., García-Olmo D.C. and Kramer K. (2011). *Effects of wire-bottom caging on heart rate, activity and body temperature in telemetry-implanted rats.* Lab Anim. Oct;45(4):247-53. Epub 2011 Jun 22.
10) Jonasson K.A. and Willis C.K. (2012). *Hibernation energetics of free-ranging little brown bats.* J Exp Biol. Jun 15;215(Pt 12):2141-9.
11) Kemppinen N, Hau J, Meller A, Mauranen K, Kohila T, Nevalainen T. (2010). *Impact of aspen furniture and restricted feeding on activity, blood pressure, heart rate and faecal corticosterone and immunoglobulin A excretion in rats (Rattus norvegicus) housed in individually ventilated cages*. Lab Anim. Apr;44(2):104-12.
12) Kramer K. and Kinter L.B. (2003). *Evaluation and applications of radiotelemetry in small laboratory animals. Physiol Genomics.* May 13;13(3):197-205.
13) Marks A., Vianna D.M. and Carrive P. (2009). *Nonshivering thermogenesis without interscapular brown adipose tissue involvement during conditioned fear in the rat.* Am J Physiol Regul Integr Comp Physiol. Apr; 296(4):R1239-47.

14) Morton D.B., Hawkins P., Bevan R., Heath K., Kirkwood J., Pearce P., Scott L., Whelan G. and Webb A; (2003). *Refinements in telemetry procedures. Seventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, Part A.* Lab Anim. Oct;37(4):261-99.
15) Newsom D.M., Bolgos G.L., Colby L. and Nemzek J.A. (2004). *Comparison of body surface temperature measurement and conventional methods for measuring temperature in the mouse*. Contemp Top Lab Anim Sci. Sep;43(5):13-8.
16) Nicholson A., Malcolm R.D., Russ P.L., Cough K., Touma C., Palme R. and Wiles M.V. (2009). *The response of C57BL/6J and BALB/cJ mice to increased housing density*. J Am Assoc Lab Anim Sci. Nov;48(6):740-53.
17) Ray M.A., Johnston N.A., Verhulst S., Trammell R.A. and Toth L.A. (2010). *Identification of markers for imminent death in mice used in longevity and aging research.* J Am Assoc Lab Anim Sci. May;49(3):282-8.
18) Sharp J., Zammit T., Azar T. and Lawson D. (2003). *Stress-like responses to common procedures in individually and group-housed female rats.* Contemp Top Lab Anim Sci. Jan;42(1):9-18.
19) Stokes W.S. (2002). *Humane endpoints for laboratory animals used in regulatory testing.* ILAR J. 43 Suppl:S31-8.
20) Trammel R.A. and Toth L.A. (2011). *Markers for predicting death as an outcome for mice used in infectious disease research.* Comp Med. Dec; 61(6), 492-498.
21) Trammel R.A., Cox L. and Toth L.A. (2012). *Markers for heightened monitoring, imminent death, and euthanasia in aged inbred mice.* Comp Med. Jun;62(3):172-8.
22) Turner PV, Kloeze H, Dam A, Ward D, Leung N, Brown EE, Whiteman A, Chiappetta ME, Hunter DB. (2012). *Mass depopulation of laying hens in whole barns with liquid carbon dioxide: evaluation of welfare impact.* Poult Sci. Jul;91(7):1558-68.
23) Van Loo P.L., Kuin N., Sommer R., Avsaroglu H., Pham T. and Baumans V. (2007). *Impact of 'living apart together' on postoperative recovery of mice compared with social and individual housing.* Lab Anim. Oct;41(4):441-55.
24) Vianna D.M. and Carrive P. (2005). *Changes in cutaneous and body temperature during and after conditioned fear to context in the rat.* Eur J Neurosci. May;21(9):2505-12.
25) Williamson E.D., Savage V.L., Lingard B., Russell P. and Scott E.A. (2007). *A biocompatible microdevice for core body temperature monitoring in the early diagnosis of infectious disease.* Biomed Microdevices. Feb;9(1):51-60.

INFRARED THERMOGRAPHY IN LABORATORY ANIMALS

AUGUSTO VITALE

Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Roma, Italy

ABSTRACT

The level of welfare in captive non-human primates can be investigated using relatively non- invasive behavioural and physiological measures. In the first case, the quality of social life and the presence of behavioural stereotypies can offer important information. In the case of physiology, level of stress can be investigated measuring the presence of stress hormones in urine, faeces and saliva. Future developments include the application of infrared thermography to obtain an indirect measure of the psychological condition of an animal in a particular environmental context.

Key words

Animal behavior, Animal welfare, Behavioural parameters, Physiological parameters, Monkeys, Non-human primates.

MEASURING WELFARE OF NON-HUMAN PRIMATES USED IN LABORATORY RESEARCH

The number of non-human primates (NHP) utilised in Europe in basic, biomedical and toxicological research is around 11,000 individuals, and it represents a fraction of the total of all animals used in the 27 Member States annually (at the moment estimated to be around 1 million individuals). However, NHP occupy a special place in the general issue of animal welfare. It suffices to say that in the recent Directive 2010/63/EU, on the protection of animals used in experimental procedures, the word “primates” occurs more often in the text than the word “sufferance” (EEC, 2010).

The reason has mainly to do with the phylogenetical closeness of NHP with humans, and the consequent supposed similarities in the capability of experiencing pain and sufferance. Therefore, whatever the reason, the welfare of NHP in captivity is a topic of special interest.

We will now briefly review different behavioural and physiological indicators of the degree of welfare of NHP, with a particular focus on relatively non-invasive measurements. These indicators are treated here separately but, as matter of fact, behavioural and physiological indices should be considered together to present a more comprehensive and informative idea on the state of welfare of a particular individual. Variations in behavioural and physiological parameters are quite normal, but more extreme variations outside the boundaries of normal range are indices of a change in the state of the individual’s degree of welfare. This consideration is valid for all of the different species and for NHP as well.

Behavioural measures

The expression of behaviour by a particular individual depends both on intra- and inter-specific factors. It follows that it is of crucial importance for a proper behavioural evaluation of welfare to have a solid knowledge of the species-specific normal characteristics of the animal, as well as his/her individual traits and history.

Having said that, for an assessment of welfare of captive animals, it is more useful to compare the behaviour of an individual before and after a particular treatment, or in the case of particular environmental events, that is, using the animal as his/her own control. This methodology is more satisfactory than a comparison with wild counterparts. It is true that species-specific characteristics are of particular relevance. However, certain behavioural needs could have been changed, due to generations in captivity. For example, common marmosets (*Callithrix jacchus*) retain anti-predator displays and reactions in captivity, but are less ready to work for food when given the choice (Manciocco personal communication), where foraging behaviours are consistent part of their wild ethogram (Lazaro-Perea *et al*, 1999). Therefore, the understanding of social organisation, feeding ethology, and general natural adaptation are essential to better provide an acceptable degree of welfare for NHP in captivity, as well as for other animals. In this sense, considerations on time-budgets are particularly relevant, but common sense must be also part of the evaluation of the captive ethogram. The important point is that any variation between captive and wild time-budgets must be considered against any signs of stress or distress by the captive individuals.

Among the different behavioural parameters to be considered, vocalisations can be very informative for investigating both the physical and emotional state of an animal in general, and of NHP in particular. Vocalisations can be recorded remotely, without interfering with the animals, and can provide a continuous tracking and development of the emotional state of a particular individual to varying environmental changes and treatments.

Play behaviour is thought to vary with environmental conditions and health status, both social and physical, of the colony(Martin and Caro, 1995). It is safe to state that the amount and quality of play behaviours reflect the emotional status of the animals involved(Boissy *et al*, 2007). The presence of play behaviours most probably indicates a good psychological state in the colony, and it represents a general status of good welfare. Play is a sort of behavioural "luxury", and it shows when all of the others primary needs are satisfied (Jensen, 1998).

The quality and quantity of social interactions can give an idea of the emotional condition of a particular group of captive NHP and, without doubt, to be kept socially is a *must* for these animals (Olsson and Westlund, 2007). For example, marmosets separated from the pair-mate for about an hour, once reunited, significantly increased the amount of allo-groming and affiliative interactions (Shepherd and French, 1999). This observation strongly suggests the importance of social housing, and isolated individuals frequently show impoverished immunological functions. (Ladenslauger *et al*, 1990; Gust *et al*, 1992).

However, it must be noted that over-crowding can also cause stress and suppress immunological defence(Plowman *et al*, 2005). In particular, in NHP species where social dominance is present, like in rhesus (*Macaca mulatta*) or cynomolgous macaques (*Macaca fascicularis*), subordinates could be at risk, if not given shelter and possibility to feed away from the dominant individuals.

Allo-grooming is a distinct feature of social relationships in NHP. A change in frequency and intensity of allogrooming could indicate the insurgence of a stressful situation. At the same time, allo-grooming can also indicate a correct social setting, where normal social relationships are in force. Experienced staff can, with knowledge of the general pattern of behaviour of that particular colony, establish whether a change from baseline of the allo-grooming activity is a sign of decreased welfare or not.

In some cases, behavioural patterns can clearly indicate poor welfare. For example, captive animals sometimes perform behaviours that appear to be functionless, if not detrimental. These behaviours include, for example, locomotor stereotypies as well as self-biting and/or hyperactivity (Bellanca and Crockett, 2002, Lutz *et al*, 2003. A stereotypy has been defined as "a behaviour pattern that is repetitive, invariant and has no obvious goal or function" (Mason, 1991). When stereotypical behaviours are manifested, it is indicative that the animal is experiencing an aversive environmental condition. This consideration is valid for all of the species kept in research laboratories. However, it has to be decided at what frequency of appearance a stereotypy has to be considered an indicator of a seriously compromised level of welfare. In this regard, research suggests that welfare can be considered very poor if stereotypical behaviours occupy more than 40% of the active time budget (Broom and Johnson, 1993).

Physiological measures

As mentioned before, in order to have a more comprehensive knowledge of the degree of welfare a particular individual is experiencing, it is always advisable to pair behavioural measures of welfare with physiological measures.

One of the first measures is body weight. Stress and suffering can cause anorexia, and then weight loss and reduced weight gain (Dettling *et al*, 2002). Monkeys can be trained to stay on a platform attached to a scale for a long enough time to register their weight (Westlund, pers. comm.).

Another non-invasive measure of welfare, in relation to physiological parameters, is hormone activities. Cortisol levels in New World monkeys are known to be much higher than the level observed in Old World monkeys and apes (including humans)(Lipsett *et al*, 1984). This is important information to keep in mind when trying to determine the level of stress experienced by captive NHP by measuring the amount of stress hormone in circulation. The classic way to obtain hormone measure is by blood samples. Using techniques of positive training, the stress imposed to the animal during blood sampling can be greatly reduced (Coleman *et al*, 2008). There are also alternative less invasive methodologies and cortisol concentrations, for example, can be measured also in faeces, urine and saliva (Davenport *et al*, 2006). In particular, with salivary cortisol, changes in the concentration of cortisol can be observed after five minutes from the appearance of the potential environmental stressor(Tiefenbacher *et al*, 2003). In marmoset stressors can be represented by isolation or unstable social settings, and saliva samples can be collected by the use of cotton swabs, after an adequate period of training (Tiefenbacher *et al*, 2003). Because the animal less stressed by this methodology, in comparison for example to blood sampling, it is likely that salivary cortisol is a more reliable method(Buchanan-Smith, pers.comm.).

The measurement of the variation of external temperature as a way to determine the emotional state of NHP is still in its infancy, but it appears to be a very promising technique. For example, in a study on rhesus monkeys variation in the temperature of different facial regions were measured, when presented with threatening stimuli. (Nakayama *et al*, 2005; Kuraoka and Nakamura, 2011). The authors concluded that the decrease in nasal skin temperature was related to alteration of the emotional state of the experimental subjects (Sikoski *et al*, 2007).

Conclusions

To ensure the welfare of NHP in research laboratories means to defend the quality of science, as well as the quality of life of the experimental animals. Obviously, this is true also for the other laboratory species. Non-invasive methods to determine the degree of welfare are available, and are reliable. The picture is even more complete if behavioural parameters can be paired with physiological measures.

The overall ethos should be that, whatever parameters are chosen to measure, acquiring a solid knowledge of the etho-ecological characteristics of the particular species used in a particular study is of paramount importance. In relation to this, the competence of the staff involved in the care and use of NHP in research laboratories is the most important factor affecting the degree of welfare.

REFERENCES

1) Boissy A., Manteuffel G., Jensen M.B., Moe R.O., Spruijt B., Keeling L.J., Winckler C., Forkman B., Dimitrov I., Langbein J., Bekken M., Veissier I., Arnaud A. (2007). Assessment of positive emotions in animals to improve their welfare. Physiology & Behavior, 92: 375-397.
2) Bellanca R.U., Crockett C.M. (2002). Factors predicting increased incidence of abnormal behavior in male pigtailed macaques. American Journal of Primatology, 58: 57-69.
3) Broom D.M., Johnson K.G. (1993). Stress and Animal Welfare. London: Chapman and Hall. Pp 1-217.
4) Coleman K., Pranger L., Maier A., Lambeth S.P., Perlman J.E., Thiele E., Schapiro S.J. (2008). Rhesus Macaques for Venipuncture Using Positive Reinforcement Techniques: A Comparison with Chimpanzees .Journal of American Associations of Laboratory Animals Science, 47: 37-41.
5) Davenport M.D., Tiefenbacher S., Lutz C.K., Novak M.A., Meyer J.S. (2006). Analyisis of endogenous cortisol concentrations in the hair of rhesus macaques. General and Comparative Endocrinology, 147: 255-261.
6) Dettling A.C., Feldon J., Pryce C.R. (2002). Early deprivation and behavioural and psychological responses to social separation/novelty in the marmoset. Pharmacology Biochemistry and Behavior, 73: 259-269.
7) EEC., (2010) " Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes". Official Journal of the European Communities L*276*, pp. 33-79, 2010.
8) Disponibile all'indirizzo: http://eurex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF.
9) Gust D.A., Gordon T.P., Wilson M.E., Brodie A.R., Ahmed-Ansari A., Clure H.M. (1992). Removal from natal social group to peer housing affects cortisol levels and absolute numbers of T cell subsets in juvenile rhesus monkeys. Brain, Behavior and Immunity, 6: 189-199.
10) Jensen M.B., Vestergaard K.S., Krohn C.C. (1998). Play behavior in dairy calves kept in pens: the effect of social contact and space allowance. Applied Animal Bejaviour Science, 56: 97-108.
11) Kuraoka K., Nakamura K., (2011) "The use of nasal skin temperature measurements in studying emotion in macaque monkeys". Physiology & Behavior, vol. 102, pp. 347-355, 2011.
12) Laudenslager M.L., Held P.E., Boccia M.L., Rite J., Cohen J. (1990). Behavioral and immunological consequences of brief mother-infant separation: A species comparison. Developmental Psychobiology, 23: 247-264.
13) Lazaro-Perea C., Snowdon C.T., de Fatima Arruda M. (1999). Scent-marking behavior in wild groups of common marmosets (*Callithrix jacchus*). Behavioural Ecology & Sociobiology, 46: 313-324.
14) Lipsett M.B., Chrousos G.P., Tomita M., Brandon D.D., Loriaux D.L. (1984). The defective glucocorticoid receptor in human and nonhuman primates. Recent Progeressions in Hormone Research, 41: 199-247.
15) Lutz C., Well A., Novak M. (2003), Stereotypic and self-injurious behavior in rhesus macaques: A survey and retrospective analysis of environment and early experience. American Journal of Primatology, 60: 1-15.
16) Martin P., Caro T.M. (1985). On the functions of play and its role in behavioural development. Advances in the Study of Behaviour, 15. 59-103.
17) Mason G.J. (1991). Stereotypies and suffering. Behavioural Processes, 25: 103-115.

18) Nakayama K., Goto S., Kuraoka K., Nakamura K.(2005). Decrease in nasal temperature of rhesus monkeys (Macaca mulatta) in negative emotional state". Physiology & Behavior, 84: 783-790.
19) Olsson I.A.S., Westlund K. (2007). More than numbers matter: The effect of social factors on behavior and welfare of laboratory rodents and non-human primates. Applied Animal Behaviour Science, 103: 229-254.
20) Plowman A.B., Jordan N.R., Anderson N., Condon E., Fraser O. (2005). Welfare implications of captive primate population management: behavioural and psycho-social effects of female-based contraception, oestrus and male removal in hamadryas baboons (*Papio hamadryas*). Applied Animal Behaviour Science, 90: 155-165.
21) Sikoski P., Banks M.L., Gould R., Young R.W., Wallace J.M., Nader M.A. (2007). Comparison of rectal and infrared thermometry for obtaining body temperature in cynomolgus (*Mcaca fascicularis*). Journal of Medical Primatology, 36:381-384.
22) Shepherd R.E., French J.A. (1999). Comparative analysis of sociality in lion tamarins (*Leontopithecus rosalia*) and marmosets (*Callithrix kuhli*): Responses to separation from long-term pairmates. Journal of Comparative Psychology, 113: 24-32.
23) Tiefenbacher S., Lee B., Meyer J.S., Spealman R.D. (2003). Non-invasive technique for the repeated sampling of salivary free cortisol in awake, unrestrained squirrel monkeys. American Journal of Primatology, 60: 69-75.

INFRARED THERMOGRAPHY IN LABORATORY ANIMALS

GIULIANO GRIGNASCHI[1], LUCIA BUCCARELLO[2]

[1] Mario Negri Institute for Pharmacological Research, Milano, Italy
[2] University of Milan, VESPA Departement, Italy

ABSTRACT

Among the different indicators used to assess animal welfare such as the evaluation of breeding performances, body weight and consumption of food and water, Infrared Thermography (IRT) is a a non-invasive and user friendly technique that can be useful to early identify possible states of stress in laboratory animals. We found that small differences in caging solutions can slightly affect the behavior of the animals and, possibly, their welfare even if most of the parameters observed were not modified (i.e. body weight, eyes temperature etc). However the exposure to a slightly stressful situation (novel environment) rapidly decrease the eyes temperature measured by thermograpy, confirming that it's a non-invasive and user friendly technique that can be useful to detect stressful situation in laboratory animals.

Key words

Laboratory animals , animal welfare, strain, mice, non-invasive methods, infrared thermography, IVC-systems, air injection, anxiety levels, cover level, animal level, body weight, food and water consumption, superficial temperature, bedding pushing score.

MEASURING WELFARE OF RODENTS IN LABORATORY RESEARCH

Introduction

It is well recognized that the general conditions of the animals submitted to biomedical experiments are crucial for the consistency of the results achieved and that the so-called "welfare" of laboratory animals is strictly related not only to the experimental paradigm but also to the housing conditions (Hawkins et al., 2011). Thus animals born and maintained in different cages may, in fact, behave in a slightly (but significant) different way, giving rise to different results when tested (Wahlsten, et al., 2003). Moreover, the presence in the home cage of environmental enrichment can completely modify the behavioural and immune response of the animals to different stimuli (Rasmussen, et al., 2011; Rivard, et al., 2000.

Welfare Indicators

In this particularly complex situation it is important to determine animal welfare through simple non-invasive methods that can be applied to a wide range of different situations (i.e. different cage types). Among the different indicators used to assess animal welfare, such as the evaluation of breeding performances (Grignaschi, et al., 2010, Grignaschi and Zennaro 2011), the evaluation of body weight and consumption of food and water, there is an innovative method of measuring the skin temperature through infrared thermography (IRT) to highlight stressful situations which could lead to a decrease in skin temperature due to peripheral vasoconstriction, which has already been shown in both rats and rabbits (Marks, et al., 2009; Vianna, et al., 2005; Yu & Blessing, 2001). A recent study showed that, using the thermographic technique, we can analyze the role that management systems and farming play on the physiology and behavior of rabbits (Verga, et al., 2007; Fig. 1a). In the field of laboratory animals this has led, through a study in nude mice, to detecting the effect of anesthesia by assessing whether the skin temperature of the animals undergoing this procedure returns quickly to a baseline level or not, possibly inducing some type of stress to the animals (Ludwig et al., 2007; Fornasier et al., 2010; Fig. **1b**).

Since the variations in surface temperature and alteration of the bloodstream are some of the most interesting indicators to indicate a response to a stressful situation (peripheral vasoconstriction), this technique can be a valuable tool to establish the welfare of laboratory animals.

Home Cages

The scientific community largely agrees that the welfare of laboratory animals can be greatly improved through the application of better housing systems that minimize the suffering caused to them, providing marked advantages both from an ethical and an experimental point of view. Extensive evidence in the scientific literature has shown that individually ventilated cages (IVCs) can guarantee better environmental conditions and, possibly a higher level of wellbeing in comparison with open cages (Baumans, et al., 2002; Grignaschi, et al., 2010; Oliva, et al., 2010). However, some differences between IVCs-systems does exist and, in particular, recent studies demonstrated that mice housed in different IVCs-systems, once given the choice, avoid draughts and prefer cages with air injected at the level of the lid rather than at animal level (Krohn & Hansen, 2010; Baumans, et al., 2002) independently from the number of Air Changes per Hour (ACH); providing nesting material can counteract this avoidance. Moreover, a "*cooling effect*" (particularly important in young and nude animals) can be observed in animals exposed to a direct air flow (Baumans, et al., 2002) and it is proposed that this may constitute a chronic stress (Marsella, et al., 2012).

Welfare Assessment

On the basis of this evidence, to ensure the highest state of welfare of laboratory animals, we decided to evaluate the behavioral and physiological (surface temperature) response of mice maintained in two different IVC-systems characterized by different points of air injection (cover or animal level) to determine any possible difference in the anxiety levels of the animals. Sixty four C57BL/6J and 64 BALB/c 6 wks old SPF, as pairs FELASA guidelines, female mice (Charles River, Italy) were used; the animals were maintained at 22± 2 °C and 55± 10% RH with food (Harlan Tekland 2018S diet) and water provided ad-libitum. The animals were housed 4 per cage in two different IVC cages with different air inlet solutions at cover (75 ACH) or animal (50 ACH) level and observed for 7 weeks (Fig. 2a-2b) (Procoli, 2012). All the cages contained the same amount of bedding (100 g hardwood shavings) and the cage cleaning procedure was performed after the first week of acclimatization and then once every two weeks.

The following parameters were recorded during the cage cleaning procedure:

- Body weight
- Food and water consumption
- Barbering episodes
- Surface temperature.

The position of the animals in the cage (number of animals in the front part / number of animals in the back part) and the appearance of a bedding pushing behavior was recorded once every two days always at the same time (10 am). At the end of the 7 weeks period of observation, the animals were exposed to a novel environment (open field) and the surface temperature measured.

RESULTS

Body weight gain and food consumption were not different between the animals maintained in the two IVC systems (data not shown) while BALB/c mice maintained in the cages in which the air was injected at "Animal level" (AL cages) consumed significantly more water than those maintained in the cages in which the air was injected at "Cover level" (CL cages) (Fig. 3B). Considering the position of the animals into the cages and the bedding pushing, we observed that mice in AL cages, independently from the strain, preferred to stay in the front part of the cage and tried to push the bedding in the direction of the air inlet while animals in CL cages preferred to stay in the back part of the cage without any episode of bedding pushing (Fig. 4). (Procoli, 2012).

As for the thermographic surveys carried out during the cage change procedure, there was no difference between the eyes temperature measured by the animals housed in CL cages and those housed in AL cages in both strains. However the eyes temperature of C57BL/6J mice significantly decreased when they were exposed to a new environment, independently from the cage (AL or CL) in which they have been housed before while in BALB-c mice the decrease in eyes temperature was observed only in the AL group (Fig. 5a-5b). (Procoli, 2012).

Conclusions

These data further suggests that small differences in caging solutions can really affect the behavior of the animals and, probably, their welfare; in IVC cages the air injected at a low level seem to disturb the animals, independently from the strain, pushing them to the anterior part of the cage and in the mean time the animals try to avoid the drafts by pushing the bedding in the direction of the air inlet. However, body weight and eyes temperature seems to be not affected by slightly different air-inlet solutions while the exposure to a novel environment (i.e. open field) rapidly decrease the eyes temperature measured by thermography.

Discussion

Looking for a technique that could monitor and, consequently, improve the welfare of laboratory animals, we used a new non-invasive technique, thermography, to identify possible states of stress in animals housed in different IVC systems, characterized by different air inlet. In fact, drafts generated by the air injected into the cage at animal level could cause the so called "cooling effect" that can be particularly dangerous in pups and nude mice. The surface temperatures detected by this two strains of mice was very variable since the coat performs a shielding function and the tail is difficult to detect in freely moving animals thus we analyzed the eye temperature that is more used and significant. (Ludwig et al., 2007). No differences in the eyes temperature was detected between animals housed in different IVC systems; this result, together with the lack of effect on body weight and food intake seems to indicate that the "cooling effect", if present, is not strong enough to determine significant metabolic changes in these animals.

However, behavioral data shown animals exposed to the draft generated by the air injection into the cage try to avoid it by spending their time far from the point of injection and trying to cover it (bedding pushing). In agreement with previous results obtained with thermography in rats and rabbits (Marks A. et al., 2009; Vianna ML. et al., 2005) we observed that in mice the exposure to a slightly stressful situation such as a novel environment can bring to a rapid decrease in superficial temperature probably due to peripheral vasoconstriction. These results confirm that thermography is a non-invasive and user friendly technique that can be useful to detect stressful situation in laboratory animals.

REFERENCES

1) Baumans V., Schlingmann F., Vonck M., Van Lith HA. (2002) "*Individually ventilated cages: beneficial for mice and men?* " Contemp Top Lab Anim Sci. 2002 Jan;41(1):13-9.
2) Cao L., Liu X., Lin ED., Wang C., Choi EY., Riban V., Lin B. and During MJ. (2012) "*Environmental and Genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission anf inhibition*". Cell 142, 52-64.
3) Fornasier M., Redaelli V., Tarantino A., Luzi F., Verga M. (2010). *Infrared Thermography (IRT) in nude mice: an alternative method for body temperature measurement.* In: New paradigms in laboratory animal science: a joint FELASA/Scand-LAS symposium. Helsinki, 2010.
4) Grignaschi G. and Zennaro E. (2011) *"Impact on breeding performance of three different cage change-cleaning procedures in individually ventilated cages"* Animal technology and Welfare, April 2011.
5) Grignaschi G., Corsi L., Zennaro E., Martino PA. (2010) "*Breeding performance as Welfare Indicator: a comparative study on C57BL/6J mice in three different individually ventilated caging system*" Lab Animal 2010 June; vol.10 No. 6.
6) Hawkins P., Morton D.B., Burman O., Dennison N., Honess P., Jennings M., Lane S., Middleton V., Roughan J.V., Wells S., Westwood K. (2011) "*A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement*" Lab Animal 2011; 45:1-13.
7) Krohn TC., Hansen AK. (2010)*"Mice prefer draught-free housing"* Lab Animal 2010 Oct; 44(4):370-2.
8) Ludwig N., Gargano M., Luzi F., Carenzi C., Verga M. (2007). *Applicability of infrared thermography as a non invasive measurement of stress in rabbits.* World Rabbit Science, vol. 15 (4), ISSN: 1257-5011.
9) Marks A., Vianna D.M.L., Carrive P. (2009) " *Nonshivering thermogenesis without interscapular brown adipose tissue involvement during conditioned fear in the rat*" Am J Physiol Regul Integr Comp Physiol 296: R1239–R1247, 2009.
10) Marsella G., Zennaro E., Grignaschi G. (2012) *"Reduced air changes per hour in IVCs and the impact on breeding performance"* Animal Technology and Welfare, August 2012.
11) Oliva AM., Salcedo E., Hellier JL., Ly X., Koka K., Tollin DJ., Restrepo D. (2010) "*Toward a mouse neuroethology in the laboratory environment*" PLoS One. 2010 Jun 29; 5(6):e11359.
12) Procoli S. (2012). Università degli Studi di Milano. Facoltà di Medicina Veterinaria Corso di Laurea in Scienze Biotecnologiche Veterinarie. Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare. *Tesi si Laurea: "Valutazione dell'impatto di differenti sistemi di stabulazione sui livelli di ansietà e sul benessere degli animali da laboratorio."* Relatore: Fabio Luzi; Correlatore: Giuliano Grignaschi & Veronica Redaelli. Anno Accademico 2011-2012.
13) Rasmussen S., Miller MM., Filipski SB., Tolwani RJ. (2011) "*Cage change influences serum corticosterone and anxiety-like behavoirs in the mouse*" Journal of American Association for laboratory Animal Science (2011), vol 50, No 4.
14) Rivard GF., Neff DE., Cullen JF., Welch SW. (2000) "*A novel vented microisolation container for caging animals: microenvironmental comfort in a closed-system filter cage*" Contemp Top Lab Animal Sci. 2000 Jan;39(1):22-7.
15) Verga M., Luzi F., Carenzi C. (2007) "*Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits*" Horm Behav. 2007 Jun; 52(1):122-9.

16) Vianna D. M. L. and Carrive P. (2005) *"Changes in cutaneous and body temperature during and after conditioned fear to context in the rat"* Eur J Neurosci. 2005 May;21(9):2505-12.
17) Wahlsten D, Metten P, Phillips TJ, Boehm SL 2nd, Burkhart-Kasch S, Dorow J, Doerksen S, Downing C, Fogarty J, Rodd-Henricks K, Hen R, McKinnon CS, Merrill CM, Nolte C, Schalomon M, Schlumbohm JP, Sibert JR, Wenger CD, Dudek BC, Crabbe JC. (2003) " *Different data from different labs: lessons from studies of gene-environment interaction.*" J Neurobiol. 2003 Jan; 54(1):283-311.
18) Yu YH., Blessing WW. (2001) *"Neurons in amygdala mediate ear pinna vasoconstriction elicited by unconditioned salient stimuli in conscious rabbits"* Auton Neurosci. 2001 Mar 23;87(2- 3):236-42.

Figures 1a-1b: Thermographic images of a rabbit and a nest of nude mice.

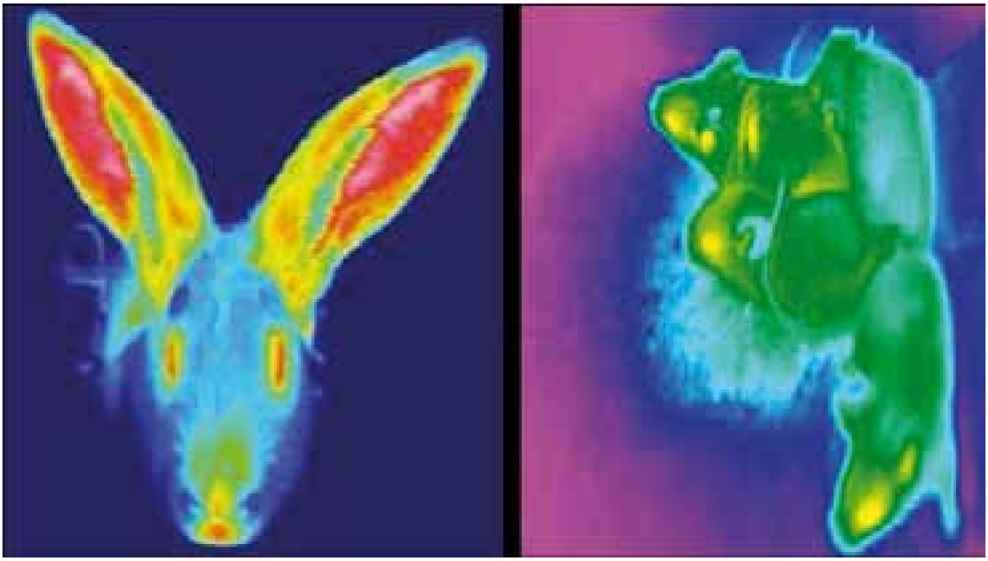
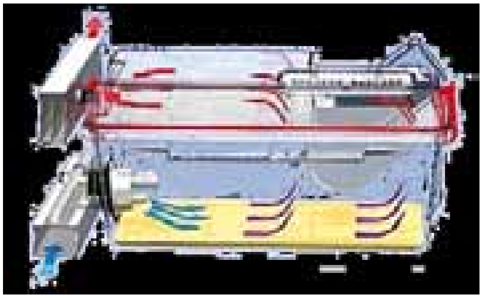


Figure 2a: IVC Cover. - Figure 2b: IVC Animal.



Figures 3a- 3b: water consumption.

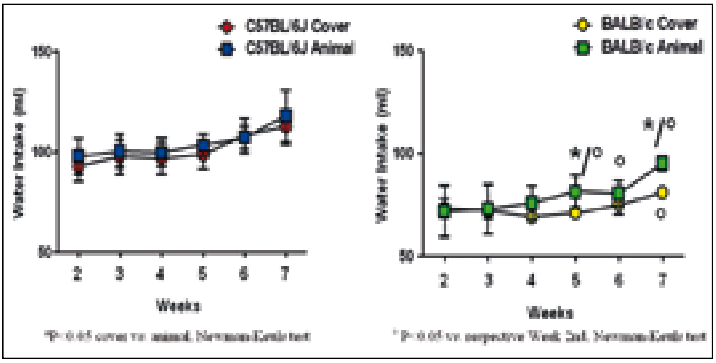
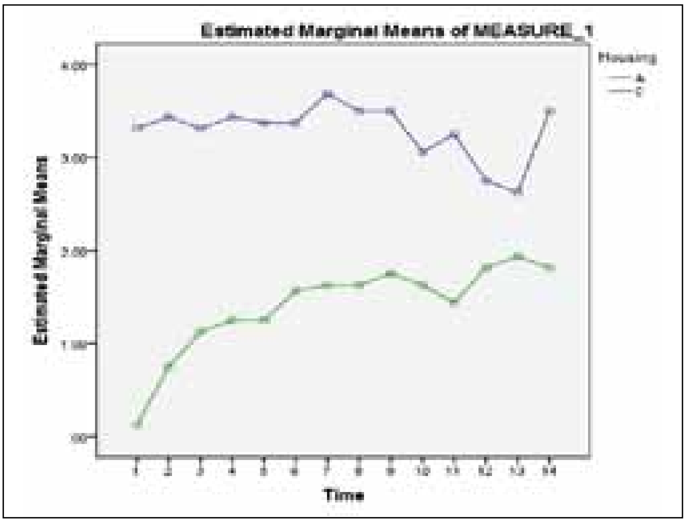
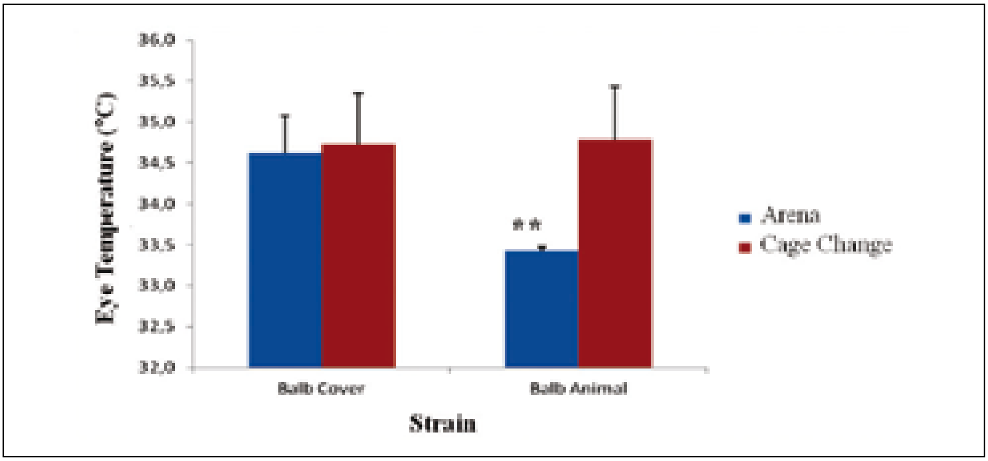


Figure 4: Position of the mice within the cage (number of animals in the front part of the cage).



*Figures 5a and b: Eye temperature of the animals housed in CL and AL cages during the cage change or in the open arena (**=P<0.01 Student's T-test).*



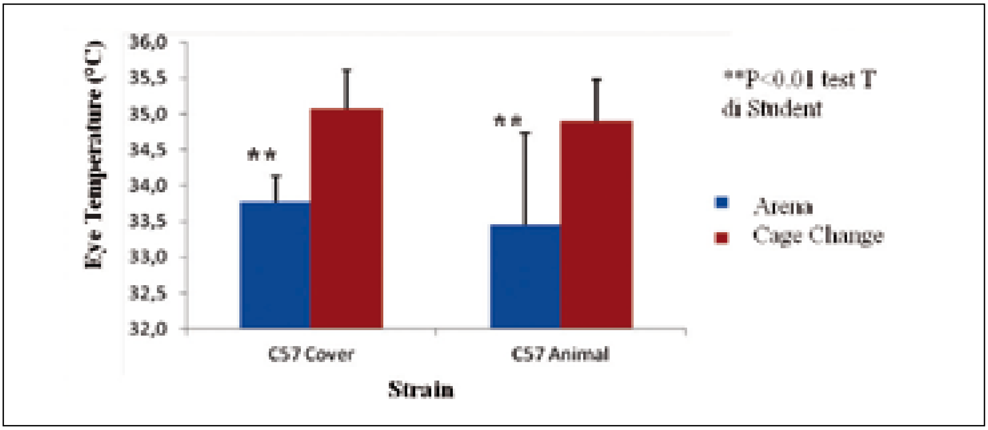
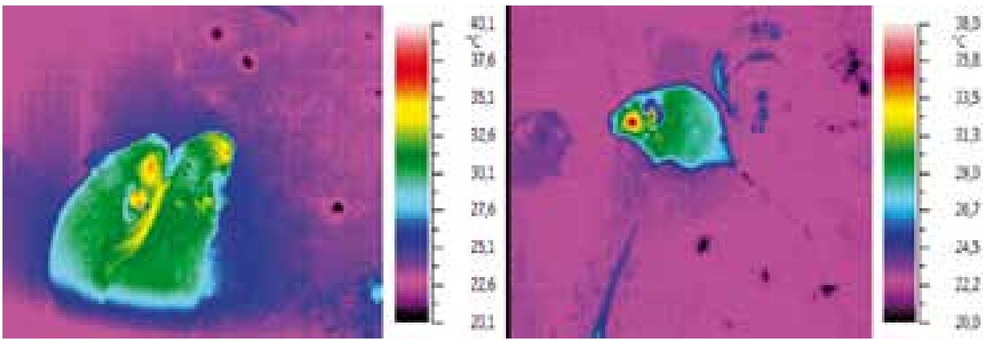


Figure 6a and b: Thermographic images of the animals.



INFRARED THERMOGRAPHY FOR DETECTION PRE-SLAUGHTER STRESS AND IMPAIRED MEAT QUALITY

LEONARDO NANNI COSTA

Dept. of Agricultural and Food Sciences (DISTAL), School of Agriculture and Veterinary Medicine,
University of Bologna, Italy

ABSTRACT

Infrared Thermography is a completely non-invasive technique that allows recording measurements of animal skin temperature on subjects difficult to reach or to approach, or moving subjects. Several characteristics such as the reliability under field conditions and the capacity to provide data instantaneously, makes its use particularly interesting at the slaughterhouse, where there is a need to detect animals that can show abnormal temperature patterns due to the pre-slaughter stress. Because of the relationship between stress and meat quality, thermal imaging before slaughter could be useful for detecting poor meat quality. Although the studies here reviewed showed the potential of infrared thermography for the detection of pre-slaughter stress and impaired meat quality, continued and closer studies of infrared thermography in this field are still warranted.

Key words

Infrared thermography, Cattle, Pig, Handling, Transport, Resting, Meat quality defects.

INTRODUCTION

Transport and handling prior to slaughter impose a severe stress on animals and can result in poor welfare and reduced meat yield and quality. Pre-slaughter practices include a series of procedures related to transport and slaughter, such as handling, loading, journey, unloading and resting in lairage pens, driving to the stunning area and use of an appropriate stunning method. During all these practices, animals experience different "physical" and "psychological" stressors" (Grandin, 1997). The physical stressors include removal of feed and water, extreme thermal condition, extreme variations in light and noise, and muscular exertion related to handling at the plant (loading and unloading), to maintaining the standing position in the truck (depending on driving quality and type of road) and to reacting to new social conditions (aggressive interaction, mount, fight, flight, etc.). The psychological stressors include unaccustomed handling and restraint as well as the exposure to new conditions in terms of social group, personnel, smells, and noises. Both physical and psychological stressors experienced during transport and slaughter lead to adaptive responses at the physiological and behavioural levels. Physiological indicators commonly used for the stress evaluation during transport and slaughter have been listed by Knowles and Warriss (2007). Variations in body and skin temperature are included in the physiological indicators because the stress can induce vascular changes in different parts of the body which are followed by changes in deep and skin temperatures (Parrot and Lloyd, 1995, Blessing, 2003). As suggested by Warriss et al. (2006a), the stimulation of the sympathoadrenal axis by stress probably leads to a redistribution of the blood and to a change in peripheral blood temperature that could be used to assess the level of stress during the stressful pre-slaughter procedures.

Kettlewell et al. (2005) stated that, aside from the effects of loading and unloading, the major stressor during transport is the thermal micro-environment within the livestock container. Cold and heat stress compromise the welfare of livestock during and after transport to the abattoir. They can affect product quality and extreme hypothermia or hyperthermia will result in death (Kettlewell et al., 2005). Despite the significance of body temperature in terms of stress experienced, it is difficult to measure this indicator under conditions of commercial transport and slaughter. The traditional apparatus for body temperature measurement, such as rectal thermometers, require the restraining of the animal and can be used only before and after transport. As an alternative, radio-telemetry devices have been used in order to record continuously body temperature during transport. In relation to pigs, ear temperature during transport was recorded by Geers et al. (1997) on pigs (20 kg live weight) with different Halothane genotype using a thermistor injected one week before the journey. After two hour of transport, a significant increase of ear base temperature was observed. Parrot et al. (1998b) recorded the deep body temperature in pre-pubertal pigs (38 kg live weight) transported for two hours, with and without indomethacin pre-treatment. The results indicated that, contrary to predictions, deep body temperature tended to fall during transport and that the effect was exaggerated by indomethacin. Recently, Mitchell et al. (2009) monitored continuously the deep body temperature of pigs during long distance transport from the UK to Spain using a data logger inserted surgically in peritoneum. The comparison between control and journey values for deep body temperature indicated no significant changes. Using similar devices, Mitchell et al. (2001) recorded deep body temperatures in calves and chickens and showed an increase of body temperature in transit. Parrot et al. (1998a) measured deep body temperature on sheep, finding an increase of 1°C after 2.5 hours of transport. From this evidence, it is not doubtful that radio-telemetry is a technique that is able to provide very relevant information on deep temperature variation during transport, but it requires a surgical implantation and it is applicable only on very few subjects who have the role of "sentinels".

In the last two decades, infrared thermography has been applied to detect surface body temperature (Stewart et al., 2005). Summarizing, this technique is based on the detection of thermal energy emitted by a body, with the signal being converted into an electronic signal, which in turn is processed by software to produce digital images. The imaging is produced using different colour scales to highlight the areas that have different temperatures, thus obtaining a representation of the thermal gradient of the body examined with an accuracy of 0.1°C. Infrared Thermography does not require any contact and is therefore a completely non-invasive technique that allows recording measurements on subjects difficult to reach or to approach, or moving subjects. Despite the opportunities offered by infrared thermography in assessing stress during animal transportation, there are few studies in the literature on the use of this technique.

Infrared thermography was used by Schaefer et al. (1988) to assess transport stress in cattle. The effect of the period of fasting and transportation of slaughter animals on infrared heat loss of beef cattle was studied. Three groups of steers and heifers were transported for 3 km, 320 km or 320 km on each of two consecutive days, and fasted for a total of 24 h, 48 h and 72 h, in the three transport treatments. It was observed that the animals subjected to the longest journey and fasting showed the lowest skin temperature. Particularly affected were body extremities such as the legs and neck and, in general, the ventral mid-section. The authors concluded that infrared thermography should be a diagnostic tool to measure stress in market cattle.

Thermal imaging of the inner surface of the ear and the blood temperature at exsanguination were used to compare the effect of fan assisted and natural ventilation of vehicles on the welfare of pigs being transported to slaughter (Warriss et al., 2006b). Irrespective of the ventilation system, ears of pigs carried on the upper deck were slightly warmer than those carried on the lower deck. A progressive increase in the temperature of ear and blood from the back to the front of vehicle was observed, probably due to the direction of the airflow from the back to the front of the vehicle. There were small differences between ear's temperatures of the pigs in the different pens, but overall the temperatures of the pigs kept in pens with fan-assisted ventilation were no lower than those of the pigs kept in pens with natural ventilation. The mean ear temperature of pigs transported with natural ventilation was 30.8°C and that of the pigs transported with fan-assisted ventilation was 31.2°C. The authors stated that the differences in ear temperature assessed by thermography reflect the physiological changes the animals were making to lose heat.

Infrared thermography (IRT) was used to detect continuously the thermometric profile of piglets during a long journey and to evaluate its relationship with the temperature inside the vehicle (Nanni Costa et al., 2012). During two journeys of 14 hours, the variation of skin temperature measured by infrared thermography was examined on a total of 12 piglets, six for each journey. More than 4000 thermal images recorded during the journeys were analysed by automatic software which considered only the maximum value of each image. The maximum value of temperature is attributable solely to the skin of animals, which has a temperature much higher than the bedding, walls and sides of the compartment. The differing thermal status of piglets recorded during the journeys are showed in Figure 1. A linear relationship between skin and environmental temperatures in the vehicle was observed with a R^2 value of 0.44 and 0.57 in the first and in the second journey, respectively. An increase of 1°C inside the vehicle increased the skin maximum temperature by 0.2°C. This evidence agrees with results obtained in an environmental chamber by Loughmiller et al. (2001). Thus, using infrared thermography it is possible to record continuously the variation of body surface temperature related to the changes of the environmental temperature inside the vehicle. This technique could be used to control transport conditions, even if there are some practical constraints due to the positioning of the camera and to the particular environment in which the measurements are carried out. The vibration of the vehicle did not influence the quality of the images recorded but might cause mechanical damage to the camera. This technique, coupled with deep temperature recording systems, will help to better understand the adaptive efforts of pigs to environmental conditions experienced during transport, in particular to extreme temperatures.

On pigs studied primarily to monitor the effect of mechanical ventilation of vehicles (Warriss et al., 2006a) the core body temperature was estimated by infrared thermography. Thermal imaging of the inner surface of the ear and the blood temperature at exsanguination were recorded. Moreover, serum creatine kinase and cortisol concentration were determined in order to assess the stress associated with pre-slaughter handling. A correlation coefficient of 0.71 ($P<0.001$) was found between ear temperature and blood temperature. Lower correlation coefficients were found between ear temperature and creatine kinase ($r=0.55$; $P<0.05$) or cortisol ($r=0.37$). Correlations between blood temperature and cortisol ($r=0.50$; $P<0.05$) or creatine kinase ($r=0.38$) were in similar direction. These relationships suggested that the hotter pigs may have been suffering more stress during pre-slaughter handling.

Although the limited number of studies have been carried out on the use of infrared thermography for detection of pre-slaughter stress, results show that this technique could be a non-invasive tool for assessing the physiological state of animals, and could be used to monitor their welfare.

In the experiment on the effect fasting period and transportation of heifers and bulls to slaughter previously described, Schaefer et al. (1988) observed that the animals that were more stressed showed not only lowest skin temperature but also darker muscle colour. Similar results were obtained by Tong et al. (1995) who used infrared thermography to detect dark beef on yearling bulls with an average liveweight of 500 kg. A group of subjects handled with lower stress (control) was compared with a treated group submitted to long fasting (24 h), mixing with unfamiliar pens and long distance transport. Dorsal thermal images were taken on both groups 3 hours before stunning. Meat quality was assessed using subjective colour scores at 24 h post mortem on the longissimus thoracis muscle at the level of 12th rib. The treated group of cattle showed a significantly lower mean temperature than the control group. The authors concluded that infrared thermography provides an alternative approach to detect animals that have been stressed and may be prone to produce impaired meat quality.

Further studies have been carried out on the use of infrared thermography to predict pork quality. Gariepy et al. (1989) evaluated the meat quality of pigs having high thermographic values before stunning. On the basis of colour and water holding capacity, the meat quality of the selected pigs was assessed and classified as PSE (pale soft, exudative), normal and DFD (dark, firm dry). Although infrared thermography was not able to predict whether the meat was PSE or DFD, it showed an increasing of meat quality defects with increasing skin surface temperature. The conclusion was that infrared thermography over the back of pigs before stunning could be a valuable, non-stressful, rapid and inexpensive method to identify pigs that could potentially yield poorer meat quality.

Schafer et al. (1989) investigated the usefulness of infrared thermography in detecting skin surface temperature differences in pigs from the three genotypes for halothane gene. Thermographic images were taken on the dorsal and external median surface of pigs in the lairage area within 1 h of arrival at the slaughter plant, and on the external and internal median of half carcasses at 45 min post mortem. Although thermographic analysis did not show any differences between genotypes, small localized areas of heat were seen on the dorsal surface of halothane positive pigs. Irrespective of genotype, pigs with cooler mean side temperatures tended to have a higher drip loss and paler colour. The authors explained this result by suggesting that the area where the thermographic measures were done could be inappropriate in terms of predicting meat quality. Moreover, they highlighted the relevance of identifying the most revealing anatomical site, the most useful temperature ranges for recording thermal images and the most adequate environmental and animal handling conditions in order to make more effective use of infrared thermography in predicting pork quality.

To determine if infrared thermography could segregate pigs based on subsequent meat quality, Lawrence et al. (2001) conducted three experiments classifying the animals as either “hot” or “normal” on the basis of the infrared temperature of the loin region recorded before slaughter. In pigs submitted to a resting time ranging from 1 h to 4 h, “hot” pigs showed less red muscle colour, while no differences were detected for pH, lightness and drip loss. In pigs rested overnight (12-16 h), differences in meat quality between “hot” and “normal” pigs disappeared. The authors stated that a recovery from previous stress before stunning reduced the effectiveness of infrared thermography in detecting poor meat quality.

In order to predict pork quality, Dikeman et al. (2003) used infrared thermography to detect pigs with asurface temperature warmer or cooler than normal. Four replications involving more than 500 pigs were carried out in a wide range of environmental temperature conditions ranging from -2°C to 26°C. “Hot” pigs (higher than 1.3 SD from the mean) showed meat with less water holding capacity in two replications with a range of temperature from 6°C to 14°C, while "cold" pigs (lower than 1.3 SD from the mean) presented the same altered meat quality or defect in the replication with a range of environmental temperature from 21°C to 26°C and misting during lairage. In the coldest replication (-2°C to -1°C) no differences in water holding capacity were detected. These results suggested that infrared thermography may allow for the detection of poor meat quality but its effectiveness was dependent on environmental conditions experienced by the pigs before stunning.

Nanni Costa et al. (2007) evaluated the possibility of using IRT on the slaughter-line for the evaluation of pork quality and ham suitability to be processed as dry-cured ham. Thermographic images were collected on 40 carcasses 20 minutes after stunning. Temperatures were recovered by processing the thermographic images of a squared area located in the center of the caudal side of ham (Figure 2). At 90 min post mortem the pH (pH1) was measured on the semi-membranous (SM) muscle of each left ham. After 24 hours of chilling at a temperature of +2°C, the measurement of pH (pHu) was repeated together with an objective colour assessment (CIE Lab system). The pH and L* (lightness) and colour values were arranged in classes in order to identify PSE, slightly PSE and normal meat. A further subjective evaluation of some characteristics of ham such as the veining defect (4-point scale, 1=none, 4= serious), the red skin defect (3-point scale, 1=none, 3=serious) and the fat cover (3-point scale, 1=insufficient, 3=excessive) was carried out on the trimmed thighs destined to be processed as Parma dry-cured-ham. The temperature of both hams were very similar and not significantly different in the pH and L* co-ordinate classes. These results are consistent with previous findings of Schaeffer et al. (1989) showing an absence of any relationship between these meat quality traits and the skin surface temperature. The veining and the red skin defect classes were not significantly related to a variation of the skin surface temperature, although in both hams there was a tendency for the latter defect (red skin) to decrease with an increase in the surface temperature. Significant differences of temperature in both hams, dependent on the fat cover score were found. An increase of temperature was found in hams with a reduced fat cover, particularly in the right hams. It is suggested that a lower thermal insulation, due to a thinner subcutaneous adipose tissue layer, might be responsible for the higher skin surface temperature. The relationship between the fat cover score of ham and the surface temperature suggests that infrared thermography could be a valuable, fast and non-invasive method to estimate its fatness. Thus, these preliminary results here showed a possible use of this technique to better select the raw hams destined to the successive dry-cured processing.

In general, the results reported in literature on the use of infrared thermography in the prediction of meat quality agree about the relevant potential of this technique, even if it is evident that several factors can affect the thermal imaging thus reducing the effectiveness of infrared thermography. Although thermal imaging of pig just before stunning or sticking seems to produce encouraging results, there is still a need to define the most effective environmental and animal handling conditions before recording the thermal images.

Infrared thermography was also considered in the prediction of carcass quality traits such as lean body mass and marbling in live cattle (Schaefer et al., 2000). Dorsal, lateral and distal images of live cattle were recorded. The image area and temperature profile within that area were used to create a prediction equation for lean yield percentage. The coefficient of determination (R^2) of lean body mass (%) predictors of dissected lean mass (%) was 0.72 for an equation which included live thermal data plus animal live weight, and 0.89 for an equation which included thermal data recorded on the live animal and on the lateral carcass image at one hour post mortem. These coefficients suggested a potential use of infrared thermography in prediction of beef carcass quality.

CONCLUSION

In the last twenty years infrared thermography has become a relevant diagnostic tool for abnormal temperature pattern measurements in human and animals. Cameras are still relatively expensive but new low-priced models with good temperature sensitivity are now available, expanding the opportunity to apply this technique into many fields of animal science. Several characteristics such as the reliability under field conditions and the capacity to provide data instantaneously, makes its use particularly interesting at the slaughterhouse, where there is a need to detect animals that can show abnormal temperature patterns due to the pre-slaughter stress. Because of the relationship between stress and meat quality, thermal imaging before slaughter could be useful for detecting poor meat quality. Although the studies here reviewed showed the potential of infrared thermography for the detection of pre-slaughter stress and impaired meat quality, continued and closer studies of infrared thermography in this field are still warranted.

REFERENCES

1) Blessing, W.W., 2003. Lower brainstem pathways regulating sympathetically mediated changes in cutaneous blood flow. Cell. Mol. Biol., 23, 527–538.
2) Dikeman, M., Spire, M., Hunt, M., Lowak, S., 2003. Infrared Thermography of Market Hogs as a Predictor of Pork Quality.National Pork Board. Research Report NPB #02-025, pp. 1-9. Http://www.pork.org/FileLibrary/ResearchDocuments/02-025-DIKEMAN.10-17-03.pdf.
3) Gariepy, C., Amiot, J., Nadai, S., 1989. Ante-mortem detection of PSE and DFD by Infrared Thermography of pigs before stunning. Meat Sci., 25: 37-41.
4) Geers, R., Puers, R., Goedseels, V., 1997. Electronic identification and monitoring of pigs during housing and transport. Comput. Electron. Agr., 17: 205-215.
5) Grandin, T., 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.
6) Kettlewell P.J., Mitchell M.A., Harper E., Villarroel E., María G., 2005. Thermal environments within transport vehicles. Proc. NJF seminar No. 361 "Transport and handing of animals a Nordic Challenge!", Alnarp, Sweden, 21-22 November 2005, pp. 24-33.
7) Knowles, T.G., Warriss P.D., 2007. Stress physiology of animals during transport. In: T. Grandin (ed.) Livestock Handling and Transport. 3rd edition. CABI Publishing, Wallingford, UK, pp. 312-328.
8) Loughmiller, J., Spire, M.F., Dritz, S.S., Fenwick, B.W., Hosni, M.H., Hogge, S.B., 2001. Relationship between mean body surface temperature measured by use of infrared thermography and ambient temperature in clinically normal pigs and pigs inoculated with Actinobacillus pleuropneumoniae. Am. J. Vet. Res., 62: 676-681.
9) Lawrence, T. E., Spire, M. F. ,Dikeman, M. E., Hunt, M. C., Hogge, S. B. , James B. W., 2001. Utilizing Infrared Thermography to Predict Pork Quality. Swine Day 2001, 131-134.
10) MiItchell, M.A., Kettlewell, P.J., Lowe, J.C., Hunter, R.R., King, T., Ritchie, M., Bracken, J., 2001. Remote physiological monitoring of livestock – An implantable radio-telemetry system. Proc. 6th International Symposium, (eds. R.R. Stowell, R. Bucklin and R.W. Bottcher), Louisville, Kentucky, USA, pp 535-541.
11) Mitchell, M.A., Kettlewell, P.J., Villaroel-Robinson, M., Harper, E., 2009. Assessing stress and welfare during transportation – the role of remote physiological monitoring. In: Proc. 60th Annual Meeting of EAAP, Barcelona, Spain, pp. 251.

12) Nanni Costa, L., Stelletta, C., Cannizzo, C., Gianesella, M., Lo Fiego, D.P., Morgante, M. ,2007. The use of thermography on the slaughter-line for the assessment of pork and raw ham quality. Italian Journal of Animal Science, 6 (Supplement 1), 704 – 706.
13) Nanni Costa, L., Redaelli, V., Magnani, D., Cafazzo, S., Amadori, M., Razzuoli, E., Verga, M., Luzi, F., 2012. Preliminary study on the relationship between skin temperature of piglet measured by infrared thermography and environmental temperature in a vehicle in transit, in: Veterinary Science. Current Aspects in Biology, Animal Pathology, Clinic and Food Hygiene, Berlin, Springer-Verlag, 2012, pp. 193 – 198.
14) Parrott, R.F., Lloyd, D.M., 1995. Restraint, but not frustration, induces prostaglandin-mediated hyperthermia in pigs. Physiol. Behav., 57: 1051-1055.
15) Parrott, R.F., Hall, S.I.G., Lloyd, D.M., 1998a. Heart rate and stress hormone responses of sheep to road transport following two different loading responses. Anim. Welfare, 7: 257-267.
16) Parrott, R.F., Bradshaw, R.H., Lloyd, D.M., Goode, J.A., 1998b. Effects of transport and indomethacin on telemetered body temperature and release of cortisol and prolactin in pre-pubertal pigs. Res. Vet. Sci., 64: 51-55.
17) Schaefer, A.L., S. D. M. Jones, A. C. Murray, A. P. Sather, and A. K. W. Tong. 1989. Infrared thermography of pigs with known genotypes for stress susceptibility in relation to pork quality. Can. J. Anim. Sci. 69:491–495.
18) Schaefer, A.L., S. D. M. Jones, A. K. W. Tong, and B. C. Vincent. 1988. The effects of fasting and transportation on beef cattle. 1. Acid-base-electrolyte balance and infrared heat loss of beef cattle. Livest. Prod. Sci. 20:15–24.
19) Schaefer, A.L., Tong, A.K.W., Robertson, W.M., 2000. Carcass production: the prediction of lean body mass and marbling in live cattle. Canadian Meat Science Association, News December 2000, pp.9-11.
20) Stewart M., Webster J.R., Schaefer A.L., Cook N.J., Scott S.L., 2005 Infrared thermography as a non-invasive tool to study animal welfare. Anim. Welfare, 14, 319-325.
21) Tong, A.K.W., Schaefer A.L., Jones, S.D.M., 1995. Detection of poor quality beef with infrared thermography. Meat Focus Int. 4:443–445.
22) Warriss, P.D., Pope, S.J., Brown, S.N., Wilkins, L.J., Knowles, T.G., 2006a. Estimating the body temperature of groups of pigs by thermal imaging. Vet Res., 158: 331-334.
23) Warriss, P.D., Brown, S.N., Knowles, T.G., Wilkins, L.J., Pope, S.J., Chadd, S.A., Kettlewell, P.J., Green N. R., 2006b. Comparison of the effects of fan-assisted and natural ventilation of vehicles on the welfare of pigs being transported to slaughter. Vet. Rec., 158: 585-588.

Figure 1. Thermal images of piglets recorded in transit.

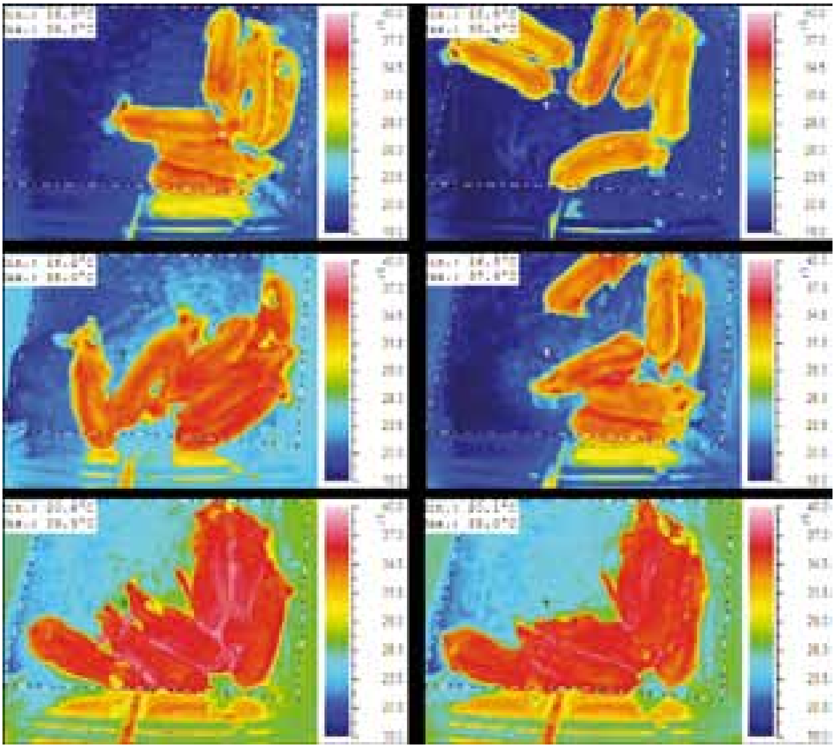
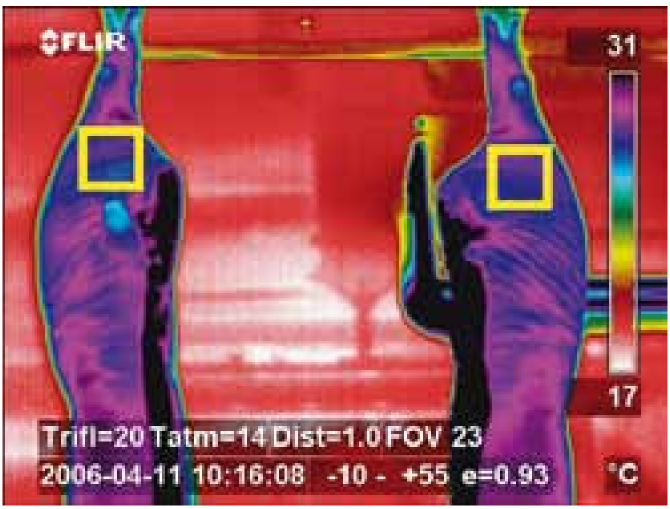


Figure 2. Thermographic image of a split carcass and the squared areas located in the centre of the caudal side of ham.



APPLICATIONS OF THERMOGRAPHY IN REPTILE BIOLOGY AND MEDICINE: UNDERSTANDING THERMOREGULATORY STATUS AND REQUIREMENTS USING THE MODEL OF MEDITERRANEAN TORTOISES (*TESTUDO SP.*)

NICOLA DI GIROLAMO, PAOLO SELLERI

Veterinary freelancer, Clinica per Animali Esotici, Centro Veterinario Specialistico, Roma, Italy

ABSTRACT

Theoretically all aspects of the behavior and physiology of ectotherms are sensitive to the body temperature, including locomotion, immune function, sensory input, foraging ability, courtship, and rates of feeding and growth, Therefore, environmental temperature is a critical parameter in the medical management of each ectotherm. Terrestrial chelonians, as the Mediterranean tortoises (Testudo sp.), mainly rely in their basking behavior to regulate their body temperature. The application of thermography to study the environment where these animals live, bask and thermoregulate, as well as to study the individual temperature, is warranted.

Key words

Reptiles, Infrared thermography, Tortoises, Temperature, Incubation, Anesthesia, Pharmacokinetics.

The processes that regulate the acquisition and allocation of resources among the competing demands of growth, survival and reproduction occupy a central place in evolutionary ecology (Candolin 1998; Ghalambor & Martin 2001; Ihara 2002). In this context, body temperature is perhaps the most important ecophysiological variable affecting the performance of ectotherms.

Maintaining a constant body temperature, through metabolic heat production, is an ecological adaptation of endotherms that remains active over a wider range of ambient temperatures (Kemp, 2006). Basically endotherms show physiological responses to environmental changes, primarily involving the initiation or curtailment of metabolic heat production. By contrast, ectotherms present behavioral and, to a lesser degree physiological, adaptation directed to the maintenance of appropriate body temperatures (Vitt and Caldwell, 2009). This means that if an ectotherm's body temperature shifts away from the set-point temperature, which is sensed by a hypothalamic "thermostat" (Rial et al., 2010), the animal moves, changes orientation, or changes posture to effect heat gain or loss (Figure 1). Theoretically all aspects of the behavior and physiology of ectotherms are sensitive to body temperature, including locomotion, immune function, sensory input, foraging ability, courtship, and rates of feeding and growth (Angilletta et al., 2002).

It seems clear, from the above publications, that environmental temperature is a critical parameter in the physiological management of each ectotherm. Terrestrial chelonians, as the Mediterranean tortoises (*Testudo* sp.), mainly rely on their basking behaviour to regulate their body temperature (Figure 2). Therefore, the application of thermography to study the environment where these animals live, bask and thermoregulate, as well as to study an individual's temperature, is warranted.

TEMPERATURE AND INCUBATION

Temperature is a fundamental parameter in the tortoises' ecology from before hatching. The sex of many reptiles is determined by the temperature experienced by the egg during the incubation period (Bull and Vogt, 1979). The sex ratio of chelonians presenting temperature-dependent sex determination is a fundamental parameter in population dynamics. Thus incubation temperature is a parameter considered in the design of conservation programs. In fact, conservation measures of free-ranging tortoises may vary, and include suggestions that in some cases eggs may be collected and incubated, with hatchlings released in the wild (Burke et al., 1996). Testudinidae specimens as *Testudo graeca, T hermanni, Gopherus agassizii* and *G polyphemus* present a temperature-dependent sex-determination with almost all hatchlings being female when incubated between 31-33°C depending on the species (Pieau, 1975; Spotila et al., 1994; Eendebak, 1995; Burke et al., 1996).

Besides sex determination, many studies have reported that incubation temperature influences the hatchling attributes of reptiles, including turtles. The range of these effects is diverse and can include determining a hatchling's, body shape, colouring, size, amount of yolk converted to tissue during embryonic development, locomotor performance, and behavior (Booth et al., 2004). Considering that variation in these traits is most likely to have consequences for the hatchling's fitness, a study was undertaken (Booth et al., 2004) in two chelonian species to evaluate whether incubation temperatures affect sex, size, amount of yolk material converted to hatchling tissue, swimming performance, post-hatch survival, and post-hatch growth. Most variables were positively influenced by higher incubation temperatures. In Brisbane river turtles, the most significant influence of incubation temperature in terms of fitness outcomes was its effect on post-hatch growth rate: hatchlings incubated at 30.8°C hatched earlier and grew faster than hatchlings from 24 and 27.8°C (Booth et al., 2004). Nevertheless, the advantages of having faster growth rates in chelonians are often discussed. Although in free-ranging individuals a smaller body size could penalize tortoise fitness (O'Brien et al., 2005; Di Girolamo et al., 2011), in captive individuals faster growth rates are anecdotally linked to several pathological conditions including obesity, high mortality, gastrointestinal illnesses, renal diseases, pyramiding (i.e. an abnormal growth of the scutes frequently observed in tortoises), fibrous osteodystrophy or metabolic bone disease (Ritz et al 2010). Pyramiding is more frequent in faster growing individuals (Gramanzini et al., 2013) and it has been associated with a reduction in life expectancy in Mediterranean tortoises (Ritz et al., 2012). In the authors' clinical experience, tortoises presenting faster growth rate are often referred for reproductive disorders (e.g. dystocia). In support of this experience-based finding is the fact that sexual maturation in freshwater turtles is size-dependent and not age-dependent (Cann, 1998). It is presumed that the sooner sexual maturity is achieved, the greater number of breeding seasons is experienced, thus increasing the life-time reproductive output (Booth et al., 2004). Although in free-ranging individuals, as well as in conservational programs, an earlier sexual maturity may be advantageous, it is also possible that individuals breeding prematurely present a lower life-expectancy due to reproductive problems. Long term clinical studies are needed to adequately address this question.

It should be noticed that in Mediterranean tortoises congenital malformations, e.g. dichepalism (Palmieri et al., 2013), microphthalmia, polydactyly, are not rare (Figure 3). Most of the congenital abnormalities have been linked to the environment in which the fertilized egg developed and appear to be related to abnormal incubation conditions during early embryonic development, especially temperatures that are too high (Palmieri et al., 2013). Newman (1917) stated that such abnormalities may be induced by a temporary cessation or radical retardation in the development of the fertilized ovum at a critical stage. Sudden changes in incubation temperature and an occasional period of anoxia were cited as possible factors. However, the majority of such abnormalities is sporadic and have not been studied in sufficient depth to be fully understood (Frye, 1991).

TEMPERATURE AND CIRCANNUAL ACTIVITY

Tortoises avoid cold temperatures in the winter by using underground cover sites (hibernacula), which generally consist of burrows or dens. Hibernacula usually have higher temperatures than the open environment during the winter and provide substantial buffering from the daily temperature fluctuations present in the environment. Thus, hibernacula provide tortoises with protection from potentially lethal temperatures in winter (Bailey et al., 1995).

Although reports of mortality rates of chelonians during hibernation are sparse in the current literature, one report (Lawrence, 1988) suggest that problem associated with hibernation accounted for 84.5% of the death of Hermann's tortoises in the United Kingdom. In temperate climate zones winter mortality of tortoises is definitely lower (Di Girolamo et al., 2011). Phenotypical factors have been demonstrated to be associated with mortality in several reptile species. Among those factors, in lizards, the loss of the tail has been associated with an increased mortality during hibernation (Bauwens, 1981). This is not unexpected, considering that the tail (together with abdominal fat bodies) acts as a reservoir for adipose tissue in this species, and that during hibernation there is a consumption of lipids especially from the tail (Avery, 1970). Another relevant factor for predicting hibernation survival in chelonians is the body size: the so-called "size-specific" winter mortality which has been observed in freshwater turtles (Bodie et al., 2000) and a similar mortality, associated with the smallest individuals in Hermann's tortoises (Di Girolamo et al., 2011). As Bodie et al. (2000) insightfully suggest, the winter-kill may be a selective force on body size and of a possible adaptive mechanism for Bergmann's Rule.

Although a role of phenotypical factors, and in this case of body size, upon survival of Mediterranean tortoises has been observed, the environmental temperature obviously play a key role in winter survival.

During clinical practice it is mandatory to provide accurate information to owners regarding the risks and the benefits of hibernation. While the benefits of hibernation are considered throughout chelonian medical literature, the risks of hibernation are difficult to be assessed objectively.

A technique for analyzing the temperatures to which hibernating tortoises are exposed could assist the practitioner in an objective evaluation of appropriate hibernacula for this species. In this regard, thermography yields a more immediate picture than thermometry. In fact, tortoises, depending on the latitudes, may leave their hibernacula on sunny days during the winter season (DeGregorio et al., 2012). Therefore an image that describes the temperature of a garden enables identification of key locations in which tortoises should be hibernated.

TEMPERATURE AND VITAMIN D SYNTHESIS

Besides its intrinsic role in the physiological activity of the individuals, temperature plays a key role in the vitamin D pathway (Holick et al., 1995; Sakaki et al., 2005). The primary function of vitamin D3 in vertebrates is maintenance and regulation of calcium homeostasis (Holick et al., 1995). Vitamin D3 aids bone mineralization via increasing uptake of calcium from the intestinal tract (Omdahl et al., 1971; Reynolds et al., 1973). This function of vitamin D3 is especially important in captive reptiles because of the high incidence of calcium deficiency–related pathological changes (ie, metabolic bone disease) in these animals (Mader, 2006; Zotti et al., 2004). In addition, chameleons with adequate circulating concentrations of vitamin D have better reproductive success than those without (Ferguson et al., 2002). The importance of vitamin D3 in reptiles is indicated by results of studies (Ferguson et al., 2003; Karsten et al., 2009; Ferguson et al., 2010) in which captive and wild reptiles voluntarily exposed themselves to UVB radiation.

Animals can obtain vitamin D3 from food or via synthesis in the skin (Hume et al., 1927; MacLaughlin et al., 1982; Horst et al., 1984; Hoby et al., 2010). Photolysis of 7-dehydrocholesterol to previtamin D3 in skin is dependent on UV radiation between 280 and 320 nm (Holick et al,1995; Taylor et al., 1963). Previtamin D3 undergoes successive temperature-dependent isomerization steps to form vitamin D3 (Holick et al,1995; Sakaki et al., 2005). At that point vitamin D obtained from food or via synthesis is converted by sterol 25-hydroxylase to 25-hydroxyvitamin D3 in the liver (Blunt et al., 1968; Ponchon et al., 1969; Heaney et al., 2008). The active form of vitamin D (1,25-dihydroxyvitamin D3) is synthesized via 1-hydroxylation of 25-hydroxyvitamin D3 in the kidneys (Fraser and Kodicek, 1970).

Reptiles bask in UV light for thermoregulatory purposes and to increase vitamin D production (Karsten et al., 2009; Manning and Grigg, 1997). In captivity, plasma concentrations of 25-hydroxyvitamin D3 increase in basking reptiles of several species when they are exposed to UVB radiation (Ferguson et al., 2003; Hoby et al., 2010; Oftedal et ak., 1997; Gillespie et al 2000; Acierno et al., 2006 Acierno et al., 2008; Oonincx et al 2010). This response has only been identified in one terrestrial chelonian species, the Hermann's tortoise (Selleri and Di Girolamo, 2012): the results of that study supported the hypothesis that circulating 25-hydroxyvitamin D_3 concentrations in Hermann's tortoises are influenced by exposure to UVB radiation (Figure 4).

Even though direct evidence is lacking, considering the thermal activation of vitamin D in other vertebrates, an appropriate temperature range may be necessary for completing the vitamin D pathway. Thus a proper thermal gradient should be offered to tortoises to enable vitamin D synthesis through the skin. Therefore, when visiting a chelonian in which hypovitaminosis D is suspected, investigation on the presence of a UVB emitting source is probably not enough. In this sense, the application of thermal imaging may assist clinicians in the diagnosis, and researchers in the comprehension, of metabolic bone diseases.

TEMPERATURE AND DRUG METABOLISM

In reptile medicine the influence of temperature on drug metabolism has always been a source of speculation. Metabolism is increased at elevated temperatures and this could assist in controlling or killing the infectious agent (Jacobson, 1996). However the variation in body temperature may also affect the pharmacokinetics of the administered drug.

Pharmacokinetic studies comparing drug metabolism at different temperatures allow clinicians to achieve a deeper comprehension about the role of temperature. Unfortunately most pharmacological studies in reptiles (Stamper et al., 1999; Benson et al., 2003) have been performed at a constant temperature, thus avoiding a critical evaluation of the role of temperature on drug pharmacokinetics and efficacy. In some of the studies reptiles were provided with a species-specific temperature range to facilitate thermoregulation, rather than a fixed ambient temperature (Tuttle et al., 2006; Divers et al., 2010). For the purpose of this chapter it is relevant to evaluate those studies in which pharmacokinetics at different temperatures were studied. Considering the restricted number of studies that fulfill this criterion in the present literature, it is useful to consider not only studies on tortoises, but also on other reptiles.

Ball pythons presented a similar pharmacokinetic for amikacin (an aminoglycoside antibiotic used to treat different types of bacterial infections) when housed at 25 or 37°C (Johnson et al 1997). Differently, in gopher snakes (*Pituophis melanoleucus*) administered with amikacin and housed at 37°C, the volume of distribution of the antibiotic was larger and it was more rapidly cleared than those housed at 25°C (Mader et al., 1985). In Gopher tortoises acclimated at 20°C and 30°C (Caligiuri et al., 1990) the half-life of amikacin administered at 5 mg/

kg body weight (shell included) was significantly less at 30°C than in the group maintained at 20°C. The clearance rate of amikacin in the warmer acclimated tortoises was approximately twice as fast as that of the cooler acclimated tortoises. Oxygen consumption was similarly found to be approximately twice as great at the higher acclimation temperature. These data indicate that while the volume of distribution was approximately the same, amikacin remained in the colder tortoises longer because of its slower elimination. In a study (Hodge, 1978) with gentamicin in water snakes (*Nerodia fasciata*) toxic effects on the kidney were more severe at 30°C compared to 20°C (Figure 5).

Interestingly, in a recent study (Kischinovsky et al., 2013) the effect of temperature on anesthesia and recovery was described in red-eared slider *(Trachemys scripta elegans).* The authors demonstrated that while the dosage of the anesthetic given (Alfaxalone, Alfaxan-CD RTU; Jurox, Australia) had a minor effect on both duration and level of anaesthesia, temperature had profound effects. In fact, when turtles were maintained at 35°C, different doses of alfaxalone provided only a short (5–10 minutes) and light sedation, whereas at 20°C, administration of 10 mg/kg alfaxalone provided sedation suitable for short non-invasive procedures such as clinical examination, blood and biopsy sampling, etc. and animals given doubled dosage reached deep anaesthesia with good muscle relaxation, sufficient for brief surgical procedures.

This factor could significantly impact on clinical practice and should be considered as a way to reduce the dosage of anesthetics, thus decreasing their secondary effects. Nevertheless, appropriate-designed safety studies are necessary to thoroughly understand the effect of low temperature during anesthesia of reptiles.

This effect of ambient temperature on metabolism and drug toxicity must be considered when determining the most appropriate dosage and administration interval.

In conclusion, it is still not possible to make generalizations regarding the effect of environmental temperatures on pharmacokinetic parameters in reptiles: while a temperature range of 30°C may be ideal for many reptiles, there are some species that will have a lower thermal optimum range and those that will have a higher one.

APPLICATION OF THERMOGRAPHY

Although thermography has been used for diagnosis of diseases in wild and domestic animals (Dunbar and MacCarthy, 2006; Dunbar et al., 2009), in reptiles it has only been used consistently for physiological studies (Borrell et al., 2005; Tattersall et al., 2006). An insightful, although still not consistently developed application, is the study of the environment that reptiles inhabit in captivity (Fleming et al., 2001; Selleri et al., 2012). Considering that environmental thermal characteristics are crucial in incubation, life and reproduction of any ectotherms, the analysis of the thermal gradient at which reptiles are exposed is fundamental. In particular, the evaluation of reptile enclosures allows modification of the "thermal" design to be suited to individual species contributing to optimal husbandry and health (Fleming et al. 2001).

A further application of thermography is in reptile legal medicine (Selleri et al., 2012). Veterinarians are nowadays more frequently called upon to assess the well-being of reptiles. In such instances, if analysis of the thermal ranges at which animals are exposed in the vivaria is requested, thermometry may be not enough: The mere evaluation of the "cold zone" and the "basking zone" provides only superficial details on the effective thermal environment. When using thermometry a few centimetres of difference in probe placement may completely alter the results of the inspection. Thermography yields a more objective picture (Figure 6)and it would be easier to provide a legal value to such images, and consequently to such inspections.

CONCLUSIONS

- Temperature control is crucial during incubation, both to manage sex ratios in conservation programs and to avoid congenital malformations.
- Temperature can dramatically influence clinical activity, e.g. anesthesia duration and recovery.
- Temperature influences the response and duration of drugs, e.g. antibiotics, and thus should be considered when dosages are prescribed.
- Environmental temperatures should be evaluated and monitored when natural hibernation is suggested to tortoise-owners to avoid peaks of winter mortality.
- In captive housed reptiles, basking-site temperatures should be considered along with the effective environmental temperatures.
- To obtain a thorough understanding of metabolic deficiencies in a captive-raised reptile, besides nutrition and UVB-light exposure, environmental temperature needs to be carefully evaluated. The mere presence of a basking site it is not enough for most reptiles.
- Reptile medicine can benefit from the use of thermal cameras, to provide a more objective assessment of thermal gradients in reptile enclosures.

REFERENCES

1) Acierno MJ, Mitchell MA, Roundtree MK, et al. Effects of ultraviolet radiation on 25-hydroxyvitamin D3 synthesis in red-eared slider turtles (*Trachemys scripta elegans*). Am J Vet Res 2006;67:2046–2049.
2) Acierno MJ, Mitchell MA, Zachariah TT, et al. Effects of ultraviolet radiation on plasma 25-hydroxyvitamin D3 concentrations in corn snakes (*Elaphe guttata*). Am J Vet Res 2008; 69: 294–297.
3) Angilletta MJ. The evolution of thermal physiology in ectotherms. J therm biol 2002; 27: 249-268.
4) Avery RA. Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. Comp Biochem and Physiol 1970;37:119-121.
5) Bailey SJ, Schwalbe CR, Lowe CH. Hibernaculum use by a population of desert tortoises (*Gopherus agassizii*) in the sonoran desert. J Herpetol 1995;29:361-369.
6) Bauwens D. Survivorship during hibernation in the European common lizard *Lacerta vivipara.* Copeia 1981;1981:741-744.
7) Benson KG, Tell LA, Young LA, Wetzlich S, Craigmill AL. Pharmacokinetics of ceftiofur sodium after intramuscular or subcutaneous administration in green iguanas (*Iguana iguana*). Am J Vet Res. 2003;64:1278-82.
8) Blunt JW, DeLuca HF, Schnoes HK. 25-Hydroxycholecalciferol. A biologically active metabolite of vitamin D3. Biochemistry 1968;7:3317–3322.
9) Bodie JR, Semlitsch RD, Price AH. Size-specific mortality and natural selection in freshwater turtles. Copeia 2000; 2000:732-739.
10) Booth DT, Burgess E, McCosker J, Lanyon JM. The influence of incubation temperature on post-hatching fitness characteristics of turtles. Internat Congress Series 2004;1275:226–233.
11) Borrell BJ, LaDuc TJ, Dudley R. Respiratory cooling in rattlesnakes. Comp Biochem Physiol Part A 2005;140:471-476.
12) Bull JJ, Vogt RC. Temperature-dependent sex determination in turtles. Science 1979;206:1186-1188.

13) Burke RL, Ewert MA, McLemore JB, Jackson DR. Temperature-dependent sex determination and hatching success in the Gopherus tortoise (*Gopherus polyphemus*). Chelon Conserv Biol 1996;2:86-88.
14) Caligiuri R, Kollias GV, Jacobson E, McNab B, Clark CH, Wilson RC. The effects of ambient temperature on amikacin pharmacokinetics in gopher tortoises. J Vet Pharmacol Ther 1990;13:287-91.
15) Candolin U. Reproduction under predation risk and the trade off between current and future reproduction in the threespine stickleback. Proceed Royal Soc London, B 1998;265:1171-1175.
16) Cann J. Australian Freshwater Turtles, Beaumont Publishing, Singapore, 1998.
17) DeGregorio BA, Buhlmann KA, Tuberville TD. Overwintering of gopher tortoises (*Gopherus polyphemus*) translocated to the northern limit of their geographic range: temperatures, timing, and survival. Chelon Conserv Biol 2012;11:84-90.
18) Di Girolamo N, Marini E, Selleri P. Relationship between post-hibernation mortality and individual factors in Hermann's tortoises (*Testudo hermanni*). Proceedings ARAV annual conference, 2011. Poster.
19) Divers SJ, Papich M, McBride M, Stedman NL, Perpinan D, Koch TF, Hernandez SM, Barron GH, Pethel M, Budsberg SC. Pharmacokinetics of meloxicam following intravenous and oral administration in green iguanas (*Iguana iguana*). Am J Vet Res. 2010 Nov;71(11):1277-83.
20) Dunbar MR, Johnson SR, Rhyan JC, McCollum M. Use of infrared thermography to detect thermographic changes in mule deer (*Odocoileus hemionus*) experimentally infected with foot-and-mouth disease. J Zoo Wildl Med 2009;40:296-301.
21) Dunbar MR, MacCarthy KA. Use of infrared thermography to detect signs of rabies infection in raccoons (*Procyon lotor*). J Zoo Wildl Med 2006;37:518-523.
22) Eendebak BT. Incubation period and sex ratio of Hermann's tortoise, *Testudo hermanni boettgeri*. Chelon Conserv Biol 1995;1:227-231.
23) Ferguson GW, Brinker AM, Gehrmann WH, et al. Voluntary exposure of some western-hemisphere snake and lizard species to ultraviolet-B radiation in the field: how much ultraviolet-B should a lizard or snake receive in captivity? Zoo Biol 2010;29:317–334.
24) Ferguson GW, Gehrmann W, Chen T, et al. Effects of artificial ultraviolet light exposure on reproductive success of female panther chameleon (*Furcifer pardalis*) in captivity. Zoo Biol 2002;21:525–537.
25) Ferguson GW, Gehrmann WH, Karsten KB, et al. Do panther chameleons bask to regulate endogenous vitamin D3 production? Physiol Biochem Zool 2003;76:52–59.
26) Fleming GJ, Isaza R, Spire MF. Evaluation of reptile thermoregulation and enclosure design using digital thermography. Proceedings ARAV annual conference, 2001.
27) Fraser DR, Kodicek E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. Nature 1970;228:764–766.
28) Frye F. Developmental abnormalities. In: Frye FL , ed. Biomedical and Surgery Aspects of Captive Reptile Husbandry. 2nd ed. Malabar: Krieger Publishing Co, 1991:393-419.
29) Ghalambor CK, Martin TE. Fecundity survival tradeoffs and parental risk-taking in birds. Science 2001;292:494-497.
30) Gillespie D, Frye FL, Stockham SL, Fredeking T. Blood values in wild and captive Komodo dragons (*Varanus komodoensis*). Zoo Biol 2000;19:495–509.
31) Gramanzini M, Di Girolamo N, Gargiulo S, et al. Dual-energy x-ray absorptiometry as a method to evaluate the influence of dietary and environmental management on Hermann's tortoises (*Testudo hermanni*). Am J Vet Res 2013. In press.
32) Heaney RP, Armas LA, Shary JR, et al. 25-Hydroxylation of vitamin D3: relation to circulating vitamin D3 under various input conditions. Am J Clin Nutr 2008;87:1738–1742.

33) Hoby S, Wenker C, Robert N, et al. Nutritional metabolic bone disease in juvenile veiled chameleons (*Chameleo calyptratus*) and its prevention. J Nutr 2010;140:1923–1931.
34) Hodge MK. The effect of acclimation temperature on gentamicin nephrotoxicity in the Florida banded water snake (*Natrix fasciata*). Proceedings AAZV annual conference, 1978.
35) Holick MF, Tian XQ, Allen M. Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic animals. Proc Natl Acad Sci U S A 1995;92:3124–3126.
36) Horst RL, Reinhardt TA, Russel JR, et al. The isolation and identification of vitamin D2 and vitamin D3 from Medicago sativa (alfalfa plant). Arch Biochem Biophys 1984;231:67–71.
37) Hume EM, Lucas NS, Smith HH. On the absorption of vitamin D from the skin. Biochem J 1927;21:362–367.
38) Ihara Y. A model for evolution of male parental care and female multiple mating. Am Natural 2002;160:235-244.
39) Jacobson ER. Metabolic scaling of antibiotics in reptiles: Basis and limitations. Zoo Biol 1996;15:329-339.
40) Johnson JH, Jensen JM., Brumbaugh GW, Boothe DM. Amikacin pharmacokinetics and the effects of ambient temperature on the dosage regimen in ball pythons (*Python regius*). J Zoo Wildl Med 1997;28:80-88.
41) Karsten KB, Ferguson GW, Chen TC, et al. Panther chameleons, Furcifer pardalis, behaviorally regulate optimal exposure to UV depending on dietary vitamin D3 status. Physiol Biochem Zool 2009;82:218–225.
42) Kemp TS. The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. Zool J Linnean Soc 2006;147:473-488.
43) Kischinovsky M, Duse A, Wang T, Bertelsen MF. Intramuscular administration of alfaxalone in red-eared sliders (*Trachemys scripta elegans*) - effects of dose and body temperature. Vet Anaesth Analg 2013;40:13-20.
44) Lawrence K. Mortality in imported tortoises (*Testudo graeca* and *T. hermanni*) in the United Kingdom. Br Vet J 1988;144:187-195.
45) MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 1982;216:1001–1003.
46) Mader D. Metabolic Bone Disease. In: Mader D, ed. Reptile medicine and surgery. 2nd ed. St Louis: Saunders Elsevier, 2006;841–851.
47) Mader DR, Conzelman GM Jr, Baggot JD. Effects of ambient temperature on the half-life and dosage regimen of amikacin in the gopher snake. J Am Vet Med Assoc. 1985;187:1134-1136.
48) Manning B, Grigg GC. Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. Copeia 1997;1997:579–584.
49) Newman HH. The Biology of Twins (Mammals). 1917: University of Chicago Press, Chicago.
50) O'Brien S, Robert B, Tiandray H. Hatch size, somatic growth and size-dependent survival in the endangered ploughshare tortoise. Biol Conserv 2005;126:141-145.
51) Oftedal OT, Chen TC, Schulkin J. Preliminary observations on the relationship of calcium ingestion to vitamin D status in the green iguana (*Iguana iguana*). Zoo Biol 1997;16:201–207.
52) Omdahl J, Holick M, Suda T, et al. Biological activity of 1,25-dihydroxycholecalciferol. Biochemistry 1971;10,2799–2804.
53) Oonincx DGAB, Stevens Y, van den Borne JJGC, et al. Effects of vitamin D3 supplementation and UVb exposure on the growth and plasma concentration of vitamin D3 metabolites in juvenile bearded dragons (*Pogona vitticeps*). Comp Biochem Physiol B Biochem Mol Biol 2010;156:122–128.

54) Palmieri C, Selleri P, Di Girolamo N, et al. Multiple congenital malformations in a dicephalic spur-thighed tortoise (*Testudo graeca ibera*). J Comp Pathol 2013. In press.
55) Pieau C. Temperature and sex differentiation in embryos of two chelonians, *Emys orbicularis* L. and *Testudo graeca* L. In: Reinboth R, ed. Intersexuality in the Animal Kingdom. Berlin and New-York: Springer-Verlag, 1975:332-339.
56) Ponchon G, Kennan AL, DeLuca HF. “Activation” of vitamin D by the liver. J Clin Invest 1969;48:2032–2037.
57) Reynolds JJ, Holick MF, DeLuca HF. The role of vitamin D metabolites in bone resorption. Calc Tiss Res 1973;12:295–301.
58) Rial RV, Akaârir M, Gamundí A, et al. Evolution of wakefulness, sleep and hibernation: from reptiles to mammals. Neurosci Biobehav Rev 2010;34:1144-60.
59) Ritz J, Clauss M, Streich WJ, Hatt J-M. Variation in growth and potentially associated health status in Hermann’s and Spur-thighed tortoise (*Testudo hermanni* and *Testudo graeca*). Zoo Biol 2012;31:705-717.
60) Ritz J, Hammer C, Clauss M. Body size development of captive and free-ranging leopard tortoises (*Geochelone pardalis*). Zoo Biol 2010;29:517-525.
61) Sakaki T, Kagawa N, Yamamoto K, et al. Metabolism of vitamin D3 by cytochromes P450. Front Biosci 2005;10:119–134.
62) Selleri P, Di Girolamo N. Plasma 25-hydroxyvitamin D(3) concentrations in Hermann’s tortoises (*Testudo hermanni*) exposed to natural sunlight and two artificial ultraviolet radiation sources. Am J Vet Res. 2012;73:1781-6.
63) Selleri P, Redaelli V, Luzi F. Use of thermography to study environmental well-being in reptiles. Proceedings ARAV annual conference, 2012.
64) Spotila JR, Zimmerman LC, Binckley CA, Grumbles JS, Rostal DC, List AJr, Beyer EC, Phillips KM, Kemp SJ. Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. Herp Monogr 1994;8:103-116.
65) Stamper MA, Papich MG, Lewbart GA, May SB, Plummer DD, Stoskopf MK. Pharmacokinetics of ceftazidime in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. J Zoo Wildl Med 1999;30:32-35.
66) Tattersall GJ, Cadena V, Skinner MC. Respiratory cooling and thermoregulatory coupling in reptiles. Respir Physiol Neurobiol 2006;154:302-318.
67) Taylor RL, Lynch HJ, Wysor WG. Seasonal influence of sunlight on the hypercalcemia of sarcoidosis. Am J Med 1963;34:221–227.
68) Tuttle AD, Papich M, Lewbart GA, Christian S, Gunkel C, Harms CA. Pharmacokinetics of ketoprofen in the green iguana (*Iguana iguana*) following single intravenous and intramuscular injections. J Zoo Wildl Med. 2006;37:567-570.
69) Vitt LJ, Caldwell JP. Herpetology: An Introductory Biology of Amphibians and Reptiles. 3rd ed. 2009: Academic Press, San Diego.
70) Zotti A, Selleri P, Carnier P, et al. Relationship between metabolic bone disease and bone mineral density measured by dual-energy x-ray absorptiometry in the green iguana (*Iguana iguana*). Vet Radiol Ulrasound 2004;45:10–16.

*Figure 1. Ectotherms present behavioral and, to a lesser degree, physiological adaptation directed to the maintenance of appropriate body temperatures. a-b. Red tegu (*Tupinambis rufescens*) during brumation. As thermal sources were not provided, the red tegus presented a complete adaptation to the thermal environment, being almost "invisible" to the thermo-camera. c-d. Red tegu (*Tupinambis rufescens*) during activity period. The animals regulate their body temperature primarily relying on a basking site. In the picture the basking site was generated by an infrared lamp.* Images were obtained in association with Redaelli V, and Luzi R, University of Milan.

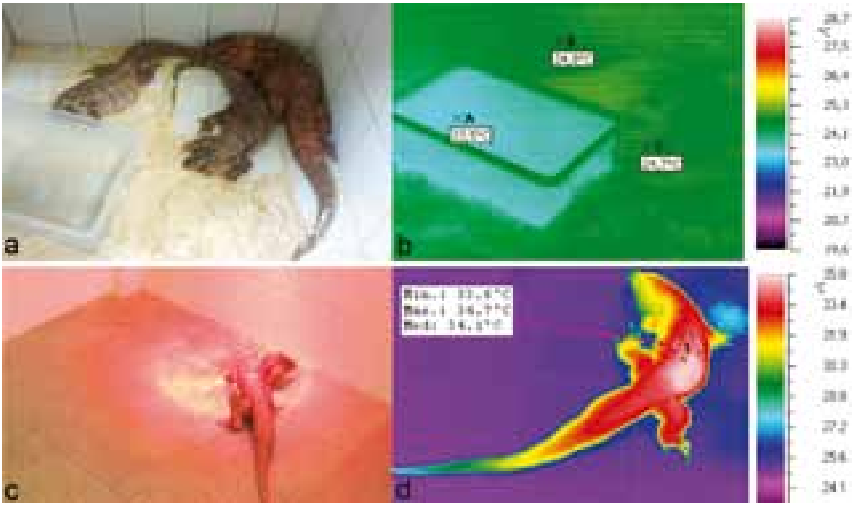


Figure 2. Hermann's tortoise basking under natural sunlight during spring. Contrarily to subterranean reptiles that gain heat through conduction, chelonians are mainly heliotherms, taking advantage of sun-generated thermal radiation to fulfill their thermal needs.

*Figure 3. Congenital abnormalities in Mediterranean tortoises. Most of the congenital abnormalities in this species have been anecdotally related to abnormal incubation conditions (especially thermal fluctuations) during early embryonic development. A. Microphthalmia in a 5.7-gram Hermann's tortoise (*Testudo hermanni*). B. Plain dorso-ventral radiography of a 17-day-old, 11-gram dicephalic (arrows) spur-thighed tortoise (*Testudo graeca ibera*), showing bifurcated vertebral column caudally to the pectoral girdle. Please notice two symmetrical gas-filled stomachs (filled arrows). The latter case was published in:* Palmieri C, Selleri P, Di Girolamo N, Montani A, Della Salda L. Multiple congenital malformations in a dicephalic spur-thighed tortoise (*Testudo graeca ibera*). J Comp Pathol 2013. In press.

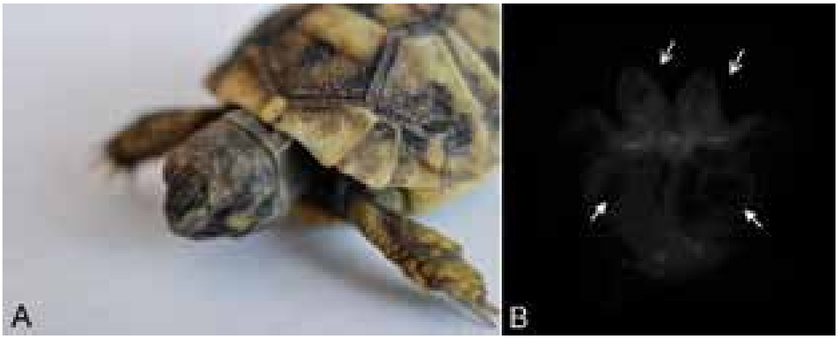


Figure 4. Amount of UVB radiation during tortoises' active season in an outdoor, sunlight-exposed enclosure where Hermann's tortoises bask. Notice that the enclosure is located in the natural geographic range of the species. Providing On-Off UVB radiation exposure as it is normally performed in captivity could not be adequate for this species. The role of temperatures and UVB light on the basking behavior in Mediterranean tortoises needs further comprehension. From Selleri P, Di Girolamo N. Plasma 25-hydroxyvitamin D3 concentrations in Hermann's tortoises (*Testudo hermanni*) exposed to natural sunlight and two artificial ultraviolet radiation sources. AJVR, 2012;73:1781-6.

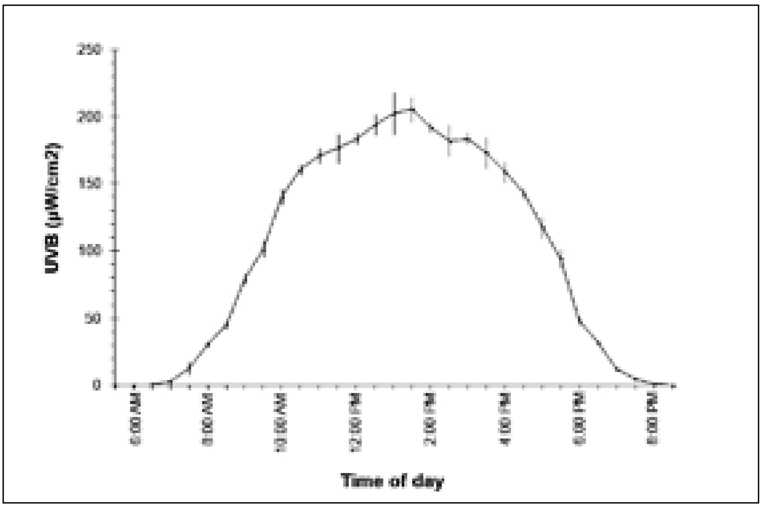


Figure 5. Thermographic imaging of a salmon Boa constrictor imperator bathing right after having acquired heat through conduction. A. Camera image. B. Thermographic image. Images were obtained in association with Redaelli V, and Luzi R, University of Milan.

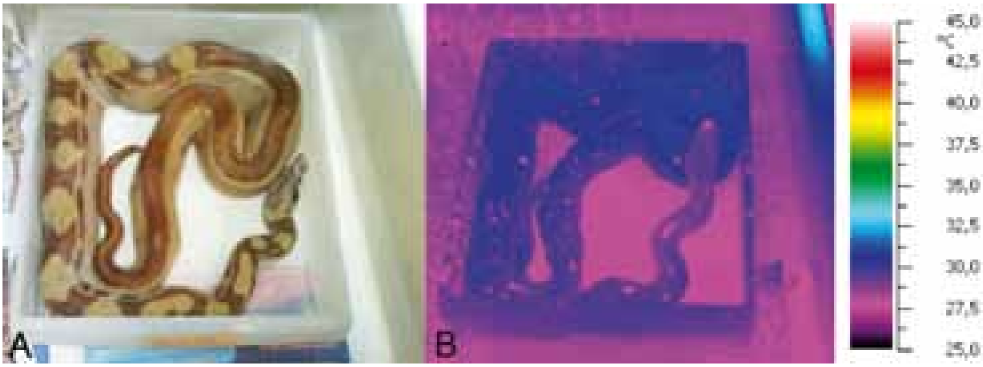
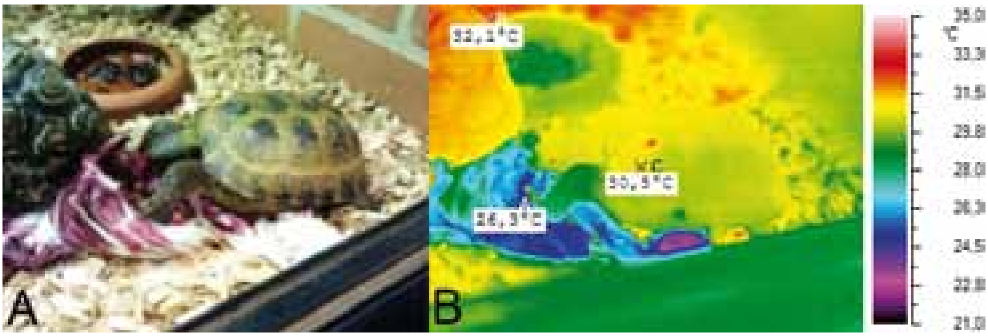


*Figure 6. Thermographic imaging of a Russian tortoise (*Agrionemys horsfieldii*) feeding in a vivarium in a shop. A. Camera image. B. Thermographic image. Please notice the complexity of thermal distributions. The chelonian was housed together with African and Mediterranean tortoises, which is strongly discouraged both for housing requirements and for infection prevention.* Images were obtained in association with Redaelli V, and Luzi R, University of Milan.



LUCIA BUCCARELLO

PhD Student

Università degli Studi di Milano
Dep. Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare

Via Celoria,10
20133 Milano, Italy
+39 (02) 503 15770 (telephone)
+39 (02) 503 17875 (fax)
lucia.buccarello@unimi.it

Lucia Buccarello is a PhD student of University of Milan, engaged in the study of neurodegenerative diseases in laboratory animals, with the view to ensure the largest state of animal welfare. After studying in a mouse model the role of oligomers in Alzheimer pathogenesis, evaluating the effect of doxycycline and the prion protein in the onset of AD, is currently studying in a mouse model the effect of different diets on the incidence of neurodegenerative diseases and animal welfare.

SIMONE CAGLIO

Physicist Freelance

via Matteotti 28
20841 Carate Brianza (MB)
Italy
+39 338 7332393
simone.caglio.78@gmail.com

Simone Caglio, graduated with a dissertation on thermography applied to archaeometric materials, is a freelance consultant for the non-invasive scientific analysis and data analysis, dealing mainly with cultural heritage both on portable media and historic architecture, from archeological to contemporary objects. He usually uses image and spectroscopic non-invasive analysis to study materials for conservation purposes, restoration and support of art historians. He has taught diagnostic techniques in several courses of restoration and master's degrees, as well as having published several articles and essays in various journals and catalogs.

NIGEL J. COOK

Research Scientist – Livestock Welfare
Office of the Chief Provincial Veterinarian
Food Safety and Animal Health Division
Alberta Agriculture and Rural Development
Lacombe Research Centre
6000 C & E Trail
Lacombe
Alberta
Canada, T4L 1W1
Tel (Office): 403 782 8057
Tel (Mobile): 403 358 2994
Fax: 403 782 8125
nigel.cook@gov.ab.ca

Dr. Nigel Cook is a research scientist with Alberta Agriculture and Rural Development, located at the Lacombe Research Centre, Alberta, Canada. His research interests focus on measurements of physiological and endocrine responses to stress and disease using minimally-invasive samples. Dr. Cook's research has focused on the measurement of corticosteroids in saliva and the diagnostic applications for stress and disease. Dr. Cook leads a research program incorporating infrared thermography in an automated, disease surveillance system for intensively housed swine. He has published over 90 scientific papers, conference proceeding and extension articles.

MAURO DI GIANCAMILLO

DVM, Associated Professor
Department VESPA
e-mail: mauro.digiancamillo@unimi.it
Via Celoria, 10
20133 Milano
Tel: 00390250317801
Fax 00390250317803
e-mail: radvet@unimi.it

Associated Professor in Veterinary Radiology and Nuclear Medicine, and Diagnostic Imaging (Università degli Studi of Milan - I); specialized in "Diseases of Small Animals"; Director of the School of Specialization in Clinical Pathology of Small Animals, University of Milan (Università degli Studi of Milan - I); Health Director of Veterinary Teaching Hospital (Lodi site - Università degli Studi of Milan - I)). Research activities: Diagnostic imaging: Computed Radiology (CR) Computed Tomography (CT) Magnetic Resonance Imaging (MRI) in small and large animal; sectional anatomy in small and large animals. More than 120 publications; 30 peer-reviewed articles indexed (PubMed, JCR, ISI WOS, Medline, CAB, Scopus).

NICOLA DI GIROLAMO

DMV
Clinica per Animali Esotici

Via Sandro Giovannini 53
00137, Roma
+39 3292003570
nicoladiggi@gmail.com

Nicola Di Girolamo is a veterinarian at Clinica per Animali Esotici, Rome. He is associate editor of BMC Veterinary Research. *He is author of several original research articles on clinical medicine of exotic animals, including description of new endoscopic procedures on chelonians. His primary interest is the application of the evidence-based medicine to exotic animal healthcare.*

ENRICO FIORE

Department of Animal Medicine,
Productions and Health
University of Padova

Viale dell'Università 16
35020 Agripolis, Legnaro (Padova) - Italy
Tel. +390498272949
Fax. +390498272954

Fiore Enrico is DVM, PhD-Student in Veterinary Sciences at the University of Padua. He has acquired and developed many researches concerning the metabolic and nutritional diseases of farm animals in livestock production, with reference to the insuline resistance, ketosis and endocrine disorders in the peripartum period. He studies the application of thermography for improved diagnosis of diseases in animal farms. He is Resident in the European College of Bovine, Health and Management (ECBHM). He is author or coauthor of 12 publications in national and international journals, books and proceedings of meetings.

MASSENZIO FORNASIER

Head of Preclinical Development Dept.
Siena Biotech S.p.A.

Strada del Petriccio e Belriguardo, 35
53100 Siena, Italy
+39 (0577) 381 421 (telephone)
+39 (0577) 381 425 (fax)
mfornasier@sienabiotech.it

Massenzio Fornasier have been working for more than 20 years in the pharmaceutical industry. He has covered the role of Named Veterinarian in big pharma and biotech companies and at present he is responsible of Animal Facility at Siena Biotech. In his career, he was involved in safety assessment of new chemical entities, in particular in safety pharmacology studies in vivo and in vitro. He developed telemetry technologies for recording of cardiovascular and respiratory parameters in freely moving animals and more recently on body temperature recordings with IR thermography. He has gained his Ph.D. in Biotechnologies applied to Veterinary Medicine at the University of Milan and is visiting lecturer at the same University. He is president of the Italian Association of Lab Animals Veterinarians (SIVAL) and Board Member of European Association of Veterinarian in Research, Industry and Education (EVERI) within the European Federation of Veterianrians (FVE).

MATTEO GIANESELLA

Department of Animal Medicine,
Productions and Health
University of Padova

Viale dell'Università 16
35020 Agripolis, Legnaro (Padova) - Italy
Tel. +390498272949
Fax. +390498272954

Gianesella Matteo - 2004: Graduated in Veterinary Medicine - University of Padua, Italy -2008: PhD at the Department of Veterinary Clinical Sciences - University of Padua, Italy -From November 2008: Researcher at the Department of Animal Medicine, Production and Health - University of Padua, Italy. He has acquired and developed many research concerning the metabolic and nutritional diseases of farm animals in livestock production, with special reference to the correlation between disorders and events occurring inside and outside the rumen environment. He's also engaged in Legal Medicine and Veterinary Legislation: he follows and develops activities related to the veterinary profession as well as the legal rules governing the tasks and functions of the veterinary surgeon to protect animal health, hygiene of livestock and livestock products. Since 2008 he was entered in SIVAR (Italian Society of Livestock Veterinarians) and since 2010 he was part of the Editorial Board and the Organising Secretariat of the scientific journal Large Animal Review, a magazine that is indexed with Impact Factor. At the University of Padua he is teaching "Legal Medicine and Veterinary Legislation". He was supervisor and correlator in many thesis of the degree course in Veterinary Medicine and of the specialistic degree course in Animal Science and Tecnologies. He has also been speaker at conferences and updating courses for veterinarians (including ECM) about metabolic and nutritional diseases of livestock and on the various aspects of the Veterinary Legislation. He is author or coauthor of 80 publications in national and international journals and proceedings of meetings in his field of expertise.

GIULIANO GRIGNASCHI

Head of Animal Care Unit
Mario Negri Institute for Pharmacological Research

Via La Masa, 19
20156 Milano, Italy
+39 (02) 39014405 (telephone)
+39 (02) 3546277 (fax)
+39 3356672612 (mobile phone)
Giuliano.Grignaschi@marionegri.it

Giuliano Grignaschi is a researcher of the Animal Care Unit, at Mario Negri Institute for Pharmacological Research, Milano (Italy). He is actually the Animal Facility Manager of the Institute; during his career he has been working not only on animal welfare, but also on neurodegenerative diseases, mechanism of action of antidepressant drugs and feeding behaviour in the Department of Neuroscience, at Mario Negri Institute. Among his responsibilities are counted as facility manager, expert in animal welfare in the field of animal experimentation, member of AISAL (Italian Association for Laboratory Animal Science) and EBRA (European Biochemical Research Association, Italian section) executive boards and visiting lecturer at the University of Studies of Milan. He also organizes training courses on animal experiments, legislation in the field of experimental and alternative methods to the use of animals in experimentation is Mario Negri institute in collaboration with other research institutes. He is author of more than 30 scientific papers, articles and books.

NICOLA LUDWIG

Ph.D.
Dipartimento di Fisica
Università degli Studi di Milano

via Celoria 16
20133 Milano - Italia
Phone +390250317486
fax +390250317422
Email: nicola.ludwig@unimi.it

Nicola Ludwig PhD. Assistant professor at State University of Milano since October 2006. Degree in Physics (University of Milan 1991), and PhD in Science for the conservation of Cultural Heritage (University of Florence 2004) with thesis on Image spectroscopy. Main topics of the research activities are: infrared thermography both applied for diagnosis of buildings and living being in particular for evapotranspiration evaluation in plant and porous building materials; infrared reflectography and visible-near-infrared spectroscopy applied characterisation of renaissance painting techniques.

FABIO LUZI

Prof. Small Animals Husbandry
Università degli Studi di Milano – Dipartimento Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare

Via Celoria 10
20133 Milano
+39 (02) 50318053 (telephone)
+39 (02) 50318030 (fax)
+39 348 5172553 (mobile phone)
fabio.luzi@unimi.it

Associated professor of Small Animal Husbandry at Veterinary Faculty (Università degli Studi di Milano). Research activities covers: management and welfare of laboratory animal; management and welfare of rabbit; thermography technique in animal production. The results of the research activities are currently applied in field implementing the management and welfare of small animals. Author of co-author of more than 170 scientific papers or communication in international and national journals and meetings.

SILVIA MARCHES

DVM, PhD student
Department VESPA
e-mail: silvia.marches@unimi.it

Via Celoria, 10
20133 Milano
Tel: 00390250317801
Fax 00390250317803
e-mail: radvet@unimi.it

PhD student in Veterinary Clinical Sciences at College of Veterinary Medicine (Università degli Studi of Milan - I). Graduated in 2011 discussing a thesis on the role of tomographic imaging and computerized radiology in detecting pulmonary nodules in dogs. Her field of interest and her doctoral project are in Diagnostic Imaging, with a special attention on the use of new imaging techniques in small animals clinic. Author and co-author of national and international conference communications.

MARIO MILAZZO

Mario Milazzo taught Archaeometry and Methodology of Applied Physics at the University of Milan. He is considered one of the founders of our country of physics applied to cultural heritage and is one of the founders of the Italian Society of Archaeometry. He has published numerous essays and articles for Italian and foreign publisher.

MALCOLM MITCHELL

Chair in Physiology and Animal Welfare
SRUC
The Roslin Institute Building
Easter Bush
Midlothian
EH25 9RG
United Kingdom
Tel (Office): 0044 (0)131 651 9353
malcolm.mitchell@sruc.ac.uk

Professor Malcolm Mitchell (BSc (first), PhD, MSB, C.Biol) holds the Chair in Physiology and Animal Welfare at SRUC. He leads a research programme focused upon the influence of stress upon the welfare of livestock. The programme has had notable success in three primary areas (1) environmental stress and animal transportation (2) environmental stress and its effects upon skeletal muscle patho-physiology in commercial poultry (3) and stress and intestinal physiology (avian) with a focus upon functional adaptations. He has published over 80 peer reviewed papers and 65 invited reviews and papers and 14 book chapters across these areas and has lead and managed more than 25 major government, research council and industry funded research projects. He currently serves on the BBSRC Panel of Experts (Animal Welfare / animal physiology) and the EFSA Panel of Experts List (Animal welfare / animal transport). He was a member of the European Food Safety Authority Working Group on the Welfare of Animals during Transport (2010). He also serves as an invited member of the DEFRA Wider Awareness Groups or the Evidence Groups and Expert Groups on Ruminants, Pig and Equidae issues (Transport and EC 1/2005 groups) and is a nominated expert (witness), advisor and consultant to Defra Legal and Trading Standards Offices (Animal Welfare and Transportation). Previously he has been an invited member of the European Food Safety Authority Working Group on the Welfare of Animals during Transport (2003) and joint author of the European Commission Report "Welfare of Animals during Transport" (2004). Also have served on the European Food Safety Authority Working Group on the Microclimate (during transport) 2003/4.

MELANIA MOIOLI

DVM, PhD student
Department VESPA
e-mail: melania.moioli@unimi.it

Via Celoria, 10
20133 Milano
Tel: 00390250317801
Fax 00390250317803
e-mail: radvet@unimi.it

PhD student in Veterinary Clinical Sciences at College of Veterinary Medicine (Università degli Studi of Milan - I). Graduated in 2010 discussing a thesis on the use of rapid prototyping and three-dimensional computed tomography in the study of medial patellar luxation in dogs. Her fields of interest and her doctoral project deal with Diagnostic Imaging, especially on the use of new imaging techniques in small animal clinic. Author and co-author of national and international conference communications.

LEONARDO NANNI COSTA

Dept. of Agricultural and Food Sciences (DISTAL)
School of Agriculture and Veterinary Medicine
University of Bologna, Italy

Via G. Fanin, 50, 40127 Bologna, Italy
+39 051 2906594 (Tel.)
+39 051 2096596 (Fax)
leonardo.nannicosta@unibo.it

Leonardo Nanni Costa is professor of animal husbandry at the University of Bologna. His research activity is mainly focussed on the effects of pre-slaughter handling on cattle and pig welfare and on the quality of meat products. Current work is related to risk analysis during transport and slaughter and to responses of Italian native pig breeds to pre-slaughter stress. He collaborates with Italian Ministry of Health on the control of road transport condition and with EFSA committee on animal welfare during transport. He is author of more than 170 scientific papers, articles and books.

VERONICA REDAELLI

Milan University – VESPA Department

Via Celoria 10
20133 Milano
+39 (02) 50318053 (telephone)
+39 (02) 50318030 (fax)
+39 338 9818139 (mobile phone)
vereda@tin.it

Degree in Physics (University of Milan 2001) with a thesis on the application of thermographic technique in the cultural heritage. In 2003 he obtained the qualification of thermographic second level technical according to UNI EN 473. PhD in Animal Production (University of Milan 2010) with thesis on the application of thermography in veterinary science. She currently lectures in training courses, seminars and conferences in the field of thermography in animal science and cultural heritage.

ROBERTO RICCA

INPROTEC IRT

Via Beethoven, 24
20092 Cinisello Balsamo (MI), Italy
Tel. +39 (02) 66.59.59.77
r.ricca@inprotec.it

Roberto Ricca started with thermography since 1977 working for major companies worldwide leader of thermography as the Swedish AGA (Agema from 1985), American Hughes and FLIR, Japanese AVIO. Since 1995, in collaboration with CND Studio of Milan, offers three courses a year of thermography and obtaining in 1998 the certification Level III in thermography offers, for the first time in Italy, courses with certification in thermography compliant to EN473. Since 2004 has lectured on thermography in the "Course in Higher Education Maintenance" at the University of Bergamo.

ELISABETTA ROSINA

Architect Polytechnic of Milan,
Department Architecture, Built Environment
and Construction engineering (ABC)

V. Bonardi 9
20133 Milano, Italy
+39 (02) 23994150 (telephone)
+39 (02) 23996020 (fax)
elisabetta.rosina@polimi.it

Elisabetta Rosina is a researcher at ABC Experimental Laboratory, Polytechnic of Milan (Italy), responsible for the mobile Laboratory unit. She is accomplished in surveys and building assessment, and in the design, planning and execution of non-destructive investigations for conservation of historic buildings. Assistant professor at Polytechnic, she teaches the classes of "Conservation" at the international Master Architectural Engineering, and "Restauro Architettonico" at the 6th faculty of Engineering. She is author of more than 150 scientific papers, articles and books.

ALLAN L. SCHAEFER

Research Scientist Animal Physiology
Lacombe Research Centre
Agriculture and Agri-Food Canada

6000 C and E Trail,
Lacombe, Alberta, Canada T4L 1W1
Ph 403-782-8100,
Fax 403-783-6120
email: al.schaefer@agr.gc.ca

Dr. Schaefer is a research scientist with Agriculture and Agri-Food Canada located in Alberta Canada. His research interests focus primarily on studying stress physiology and animal welfare particularly in the areas of using non invasive technologies. Dr. Schaefer's research has incorporated the use of infrared thermography to study transport and handling stress, early disease detection such as bovine respiratory disease and the use of infrared to monitor health and growth efficiency. Dr. Schaefer is currently also an adjunct professor at the University of Alberta and the University of Manitoba. He has published over 100 scientific journal manuscripts, book chapters and books.

PAOLO SELLERI

DMV, PhD, SpecPACS, Diplomate
ECZM(Herpetology)
Clinica per Animali Esotici

Via Sandro Giovannini 53
00137, Roma
+39 3385407051
paolsell@gmail.com

Paolo Selleri is owner and CEO of Clinica per Animali Esotici, Rome. He is a Diplomate of the European College of Zoological Medicine for the specialty herpetology. He is the past president of the SIVAE (Italian Association of Exotic Animal Veterinarians). He is author of books and several original research articles on various aspects of exotic animal medicine, ranging from anatomy to surgery. One of his major concerns is the improvement of welfare in captive-raised exotic animals.

CALOGERO STELLETTA

Department of Animal Medicine,
Productions and Health
University of Padova

Viale dell'Università 16
35020 Agripolis, Legnaro (Padova) - Italy
Tel. +390498272949
Fax. +390498272954

Stelletta Calogero: 1997: Degree in Veterinary Medicine at the University of Perugia. 1998: Qualifying examination to become Veterinary Doctor. 2003: Doctorate in Veterinary Public Health and Food Safety - curriculum: Animal Welfare. 2005: Research grant at the CRA - Italian Ministry of Agriculture. Experimental Dairy Cows farm in Porcellasco (Cremona). 2006: Researcher and assistant professor at the Department of Animal Medicine, Production and Health, University of Padova. Clinician and theriogenologist with main interest in diagnostic approaches for reproductive diseases in farm animal (Ruminant, Camelid, Horse), teaching activity on reproduction and reproductive diseases of farm animals, member of the international affairs commission of the Faculty of Veterinary Medicine of the University of Padua, board member of the Doctoral School in Veterinary Medicine (Farm Animal Reproduction) of the University of Padua, Teacher of Pathology of reproduction and Clinics in Reproduction of the farm animals for the 2009-2010, 2010-2011 and 2011/2012.
2011: Diploma of the European College of Small Ruminant Health Management.
He is author or coauthor of 120 publications in national and international journals and proceedings of meetings in his field of expertise.

STEFANO TARANTINO

DVM free practitioner, Milano

00393383748644
www.maniscalchibovini.it

Stefano Tarantino is a vet/hoof trimmer. He has been dealing with bovine lameness since 1994. As a free practitioner works in several farms in Northern Italy.
He is accomplished as a farm consultant with pharmaceutical and feed producers industries. Co-relator in several degree thesis in Faculty of Veterinary Medicine, Milano, Italy, deals with infrared diagnosis for bovine lameness as free practitioner since 2009.
He is member of Hoof Trimmers Association (USA) and of the International Society for the Study and Research in ruminant Lameness.
He is a co-author of oral communications and posters in international Congresses.

EMANUELA VALLE

DVM
University of Turin
Departemnt of Veterinary Science

Via L. Da Vinci 44
Grugliasco
To 10095
+39(011)6708856
emanuela.valle@unito.it

Emanuela Valle is a researcher at Department of Veterinary Science, University of Torino. She is author of more than 50 different publications regarding the welfare and the physiological aspects of the Horse. In 2009 she was awarded a Doctorate (PhD) by the Department of Animal Production, Epidemiology and Ecology, at the University of Veterinary Medicine of Turin. At present she is a Resident at the "European College of Veterinary and Comparative Nutrition" and works for 15% of her time in clinical veterinary medicine, 60% of her time is spent in research and 25% in teaching.

JURI VENCATO

Department of Animal Medicine,
Productions and Health
University of Padova

Viale dell'Università 16
35020 Agripolis, Legnaro (Padova) - Italy
Tel. +390498272949
Fax. +390498272954

Vencato Juri is DVM, PhD student in Veterinary Sciences at the University of Padua. He is accomplished in reproductive biotechnologies in farm animals and the use of innovative technologies, including Infrared Thermography, for the optimization of reproduction. He is a Resident in the European College of Animal Reproduction sub-speciality ruminants. He is author or co-author of more than 20 publications in scientific journals, proceedings of meetings and book chapters.

MARINA VERGA

Marina Verga taught Applied Ethology at the University of Milan.
She is considered one of the founders of our country of ethology applied to animals. Her research has focused on the relations between technologies in breeding and biotechnology, adaptation and stress in pets - Ethology applied to pets - Human-animal assisted therapy with animals - Thermography applied to animal production. She was the author of over 300 publications in national and international magazines.

AUGUSTO VITALE

Biologist
Istituto Superiore di Sanità,
Department of Cell Biology and Neuroscience
Section of Behavioural Neuroscience

Viale Regina Elena, 299
00161 Roma, Italy
+39 (06) 49902107 (telephone)
+39 (06) 4957821 (fax)
vitale@iss.it

Augusto Vitale has obtained his Ph.D. in Behavioural Ecology at the University of Aberdeen (Scotland) in 1988. He has then started to work on non-human primates the following year, collaborating with Dr. Visalberghi of the CNR in Rome, In 1991 Dr. Vitale has obtained a position as researcher in animal behaviour at Italian National Institute of Health in Rome, within the Department of Cell Biology and Neuroscience. He has been President of the Italian Association of Primatology, and is currently General Secretary for the European Federation of Primatology. His main interests are the mechanisms and evolutionary consequences of social learning, and the different aspects involved in animal experimentation, ranging from scientific to ethical factors is member of the Expert Working Groups, dealing with different legislative issues regarding the use of animals in experimental procedures in the European Community.

ALFONSO ZECCONI

Prof. Epidemiology and Infectious Diseases
Università degli Studi di Milano – Dipartimento
Scienze Veterinarie e Sanità Pubblica

Via Celoria 10
20133 Milano
+39 (02) 50318073 (telephone)
+39 (02) 50318079 (fax)
+39 3318671118 (mobile phone)
alfonso.zecconi@unimi.it

Full professor of Epidemiology and Infectious diseases at Veterinary Faculty. Research activities covers: epidemiologic studies on risk factors for mastitis (clinical and subclinical); epidemiologic studies on risk factors for Bovine Viral Diarrhoea virus and Johne's disease; development of an immunological model to assess immune status of the cows at blood and milk level; assessment of the relationship between machine-induced teat tissue changes and mastitis incidence; Characterization of S.aureus and relationship with the pathogenicity of different strains. The results of the research activities are currently applied in field implementing herd health management programs for mastitis and other infectious diseases in dairy cattle. The development of these herd health programs includes the collection of data and samples throughout the Country and organization of a large number of lectures and seminars for practitioners and farmers to implement herd health programs. Author or co-author of more than 360 scientific papers or communication in international and national journals and meetings.

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