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Review Article

Application of infrared thermography for animal health study

Sanjib Borah, Simson Soren, Kuleswan Pame, Dipankar Paul, Latika Kalita

Abstract

Infrared thermography (IRT) is an advanced, safe, non-invasive, and contactless technology used to determine the surface temperature of an object. Thermal cameras absorb the infrared radiation generated by the surface, then transform it into electrical signals, and provide a thermal image of the body's surface temperature distribution. In addition to human medicine, IRT has mostly been used in veterinary medicine for diagnostic purposes and to detect distress in an animal. However, IRT can be a very effective technique for livestock and poultry breeding research *viz*. thermoregulation, reproduction, animal welfare, etc. Before pre-clinical testing, it is crucial to intervene to reduce the stress levels in experimental animals for the betterment of the experimental outcome. Where the infrared thermography could help us to monitor and assess the health status or the stress level in addition to behavioral and physiological parameters before the pre-clinical testing might be beneficial for better outcome and reproducibility. Therefore, this manuscript reviews the recent advances in infrared thermography and its use in animal science.

Keywords animal, health, infrared thermography, stress, temperature

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Authors:
S. Borah ✉, S. Soren, K. Pame,
Lakhimpur College of Veterinary Science,
Assam Agricultural University, North
Lakhimpur-787051, Assam, India

D. Paul*
Lakhimpur College of Veterinary Science,
Assam Agricultural University, North
Lakhimpur-787051, Assam, India

*ICAR-National Dairy Research Institute,
Deemed University, Karnal-132001, Haryana,
India

L. Kalita
Kamrup Polytechnic, Baihata Chariali,
Kamrup-781381, Assam, India

✉ sbsborah06@gmail.com

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Introduction

The advancement of technology has made our lives easier, faster, and more competitive day by day. Our steps with the development of newer technologies are sometimes difficult. Much of the information is around us in different fields, making us rely on each other, realizing we know very little. A biologist needs to correlate with various other disciplines. Many engineering tools are applied in the biological field, hardly have we known the mechanism of those instruments nor the other one knows the biology. We cannot grow alone; everyone's knowledge is limited. Some may be brilliant in one area of science; however, without the other it is incomplete. Therefore, a holistic approach in various fields is growing; interdisciplinary research is advancing, opening newer technological developments. The integrated or interdisciplinary approach in research allows us to communicate with different experts from many disciplines. It is difficult but yet we love to do so for a better outcome. Interspecies communication is not unfamiliar to us, nor is the interaction between humans and animals. We may not able to understand all their communicative language, even though we understand many of their expressions of communication. Understanding behavioural expression

or vocalization helps us to care for and manage the experimental animals. It may require some time to understand them. Some of us love to have pet animals. It may be a dog or a cat. You may require some time to know them or vice versa. Your time and experiences mean a lot for better companionship. We want them to be joyful, active, and responsive to our orders/ direction/ voices. The ultimate goal of our pets is to have companionship. We provide the pet with all the necessities to cope or adjust to the given facilities. None wants their pet animal should be sad, depressed, hungry, or sick. We take care of them as much as we can. That is nothing but welfare. For example, the weaning of calves is stressful on both sides i.e., the mother and the calves. When the separation is done with the provision of seeing each other through a net, can see each other but cannot live together physically. It will help to minimize the stress level of the mother and the calves. On the other hand, if the separation is done without any strategies of seeing each other i.e., mother and calves, then the stress level will increase, and it might cause economic loss to the farmer and compromisation in the welfare system.

The complete removal of stress in the experimental animal is difficult and seemed almost impossible. Human-animal interaction has to continue during the experimentation however, providing necessary facilities to make the animal adjust to the given environments minimizes their stress level. It required monitoring and assessment of the experimental animals. The 3Rs principles, *viz*. replace, reduce, and refine, are like the pillars while planning the experimentation on the animals. If possible, replace the experimentation on animals. The second is to reduce the number of animals used for experimentation. How many animals are required statistically; can the number of animals represent the target group. It is always advisable to discuss with a statistician before reducing the number of animals. The third is refinement. Refinement of the procedures is required to minimize the pain or stress level.

The contribution of farm animals to biomedical research is significant. The farm animal can serve as a dual-purpose model. The frequency and a large amount of sampling are possible in large animals. The contribution of farm animals in the area of reproductive biotechnology is still playing a vital role in human reproduction. Artificial insemination is widely used in large animals in field conditions. The intervention of improving the success rate of artificial insemination is beneficial for humans and animals also. The infertility clinics are growing all over the world, the intrauterine insemination (IUI), and in-vitro fertilization is a regular procedure in human reproductive biotechnological intervention. The effect of different compounds and their toxicity can be studied on germ cells and embryos collected from slaughtered animals for biomedical research. The gene-editing tools and their application in farm animals, as well as the studies on the polycystic ovarian syndrome in cattle, are important for clinical studies. The pregnant sheep for the studies of intrauterine growth restriction (IUGR) is being used as an animal model. The studies on cryopreservation in different animals species and its improvement, thermal stress on farm animals, the ameliorative measures, thermoregulatory mechanism of the scrotum, fertility proteins from bull spermatozoa and its application in embryo production, Sertoli cells, Leydig cells, and spermatogonial stem cell research, etc. cannot be under-estimated. Anti-venom production using horses is a great contribution to human wellbeing.

A large percentage of biomedical research is carried out in mice due to its advantage of care and management, which serve as an animal model in various biomedical researches. The welfare of the experimental animals' does not only follows the rules but responsibilities. Their contribution in various fields of human benefit and health is noteworthy. The proper care of the laboratory or experimental animals required basic scientific knowledge about animals' behavior and physiology. The monitoring and assessment of their health are very crucial for pre-clinical studies. The requirement of suitable animal models for the preclinical studies, the design of the experimentation, the procedure, and the outcome of the experimentation have to be explained in detail for the permission for experimentation on animals. After getting permission, the grouping or selection of the animals and the health status monitoring have to be carried out carefully before the pre-clinical

testing. If the animals are not available for the studies in your institute, have to procure from other authorized institutes and then transported to your facilities followed by quarantine with proper supervision and screening of the animals for the pre-clinical studies. It should be sure that the animals were under minimum stress, disease-free, or any abnormalities or injuries before the testing. It is necessary for a better outcome and reproducibility of pre-clinical testing.

Recognition of stress in animals

The animal generally responds to the stressor by sympathomedullary pathway or hypothalamic pituitary adrenal (HPA) axis, there may be involvement of various parts of the nervous system. The sympathetic nervous system stimulation causes an increase of epinephrine and norepinephrine from the adrenal medulla resulting in increased heart rate and peripheral vasoconstriction. The peripheral vasoconstriction causes the variation in the surface temperature of the body, which can be measured by infrared thermal cameras. The increased concentration of cortisol level is shown due to hypothalamic- pituitary adrenal (HPA) axis in response to stress stimuli. If there is a continuous higher concentration of cortisol in the circulation can lead to fearfulness, anxiety, aggression, memory impairment, and depression involving many parts of the brain i.e., prefrontal, amygdala, hippocampus, etc. The recognition of stress is based on behavioral (grooming, appetite, aggressive, activity, facial expression vocalization, appearance, posture, etc), physiological (rectal temperature, pulse, respiration rate, body weight, blood profile, etc), and biochemical (corticosteroids, catecholamine, and other related hormones) parameters. The behavioral expression in response to stress stimuli required basic knowledge and understanding of animal physiology, the presence of a skillful person is necessary for observation and sometimes requires 24 hours monitoring. The behavioral or psychological expression of experimental animals is to prevent themselves from harmful effects. The pain stimuli or any visceral pain include the emotion and other centers of the brain leading to distress in animals and have to attend to as soon as possible. When the stress stimuli become distressing to animals, the outcome of the experimentation is affected. The measurement of stress is qualitative; it is difficult to quantify the level of stress. However, the measurement of stress in different possible ways gives cumulative data on the stress level in experimental animals. The behavioral and physiological parameters have to be attended by a skilled person or professional (veterinarian). The interpretation of behavioral expression is sometimes misled due to the variation between the individual, species, and breeds. The collection of blood for biochemical analysis may further increase the stress level in the animals.

When the stress stimuli cause distress to the animals, they utilized the reserved energy to cope or overcome the adverse effect of stress. The surplus energy is stored as a reserve form, utilize whenever is necessary. Reserve energy plays a significant role in the time of need. Under stressful conditions, reserve energy is being utilized. The recovery process of reserve energy back to normal requires time. The recovery process depends upon the severity of the stress. As early as possible back to normal requires strategies to minimize the stress level and duration. The intervention to minimize the stress level in experimental animals before the pre-clinical testing is essential. But how do we analyze the stress level or how do we understand the distress level of animals properly for possible intervention. Therefore, the infrared thermography might help us to monitor and assess the health status or the stress level in addition to behavioral and physiological parameters before the pre-clinical testing might be beneficial for better outcome and reproducibility.

Infrared thermography

Infrared thermography (IRT) is a non-invasive contactless measurement of body surface temperature. It captures the emissivity (radiation) of an object and converts it into a thermal image of an object, making it possible to see in our naked eye and can study the surface temperature variation of an object at every point. We know that every object radiates infrared, this can be measured by

thermal cameras. The body surface temperature varies in different physiological conditions as well as environmental conditions. The variation of the surface temperature is influenced by the peripheral circulation. The significant variation of animals' body surface temperature in some anatomical sites (eye, nose, the base of the ear, tip of the nose, coronary band in the leg, extremities, flank, lower abdomen, mammary gland, etc.) was found to be an important indication regarding the physiological stage and the health status of an individual. Therefore, analyzing the body surface temperature of an animal and validating the different anatomical sites representative of rectal temperature and physiological stage without restraining the animal for health assessment can be a promising technology. The thermal stress, distress, and the activities of the animals can be assessed by measuring the body surface using infrared thermal cameras. Many times, the restraining of animals for rectal temperature measurement or health assessment and treatment further increases the stress level. A non-invasive, contactless device is in demand for assessing the disease, reproductive health, and welfare of experimental animals. The variation of the peripheral circulation and body surface temperature was reported in various sites of the body during disease, infection, depression, anxiety, metabolic disorder, reproductive cycle, thermal stress, and also an indication of rectal temperature.

Thermography for the measurement of scrotal surface temperature and its relationship with semen characteristics is also shown to be a promising tool in the screening of good-quality bulls. The efficiency of the thermoregulatory mechanism of the scrotum/ testes between and within the species can be studied using thermography. The gradient of temperature differences between dorsal and ventral scrotal surface temperature reported a better relation of semen characteristics [1]. The impact of heat stress on scrotal surface temperature and its negative impact on semen quality was also studied on bulls using thermography [2]. The stress stimuli on animals showed lower extremities surface temperature which might be due to sympathetic mediated vasoconstriction. The increase in body surface temperature was reported in disease and visceral pain conditions in some parts of the body. The variation of body surface temperature might differ from species to species, however, proper validation considering different influential factor on thermal images have to be considered before any interpretation.

Smooth handling of experimental animals minimizes the stress level than improper handling. The infrared thermal imaging of wattle and comb in birds showed variation after applying two methods of handling i.e., Cradled and side-pinned, a significant drop of wattle and comb temperature in the side-pinned method over cradled was noticed [3]. The vasoconstriction *via* sympathetic stimulation resulted in a drop in wattle and comb temperature. These organs are an important thermoregulatory mechanism in birds. The effectiveness of the analgesic drugs on animals' experimentation might sometime show hyper analgesic when it is done on stressed animals. The tail-flick test gives a false positive response in "fear" rats which indicates the stress-induced analgesia in experimental animals [4]. The facial expression of chimpanzees showed a drop in the nose temperature under fearful conditions (conspecific sounds and fighting videos) [5]. Infrared thermography is also used in monkeys and apes to find suitable anatomical sites indicative of stress levels. The surface temperature variation is influenced by the severity of stress stimuli, pain, or visceral pain. This technology also has been validated in medical sciences for early detection of inflammation, injury, tumor growth and its progression, peripheral neuropathy, and mental disorder. Infrared thermography is most commonly used in Racehorses for screening the injury sites, disease conditions, and the welfare of the animals. The tail temperature of the rats drops significantly after stressful stimuli are detected by infrared thermography [6]. When an animal face sudden fear and stress stimuli cause an increase in cardiorespiratory responses. The measurement of the cardio-respiratory responses required interaction of human or measuring devices in the body of the animals which further increase the heart rate. The development of an algorithm for the use of contactless infrared thermography and the trajectories converting them into heart rate and respiratory rate has

been tried in pigs. This might help to record the heart and respiration rate without any contact with the animal. Detection of ear temperature by infrared thermography in the rabbit indicates the stress level [7]. The recording of body surface temperature in nude mice showed an equal result in comparison with rectal temperature and other recording devices [8]. The eye, body, and tail surface temperature of small laboratory animals indicate the possible stress level, however, the requirement of good software providing an alarm of some target surface temperature variation using infrared thermal images might be a promising tool for laboratory animal welfare system. The measurement of vaginal temperature using the infrared thermal camera during the follicular and luteal phase and its correlation with plasma progesterone is promising to detect the estrus in buffalo and pigs [9-10]. The measurement of eye temperature at early detection of viral infection and the stress in goats are reported using thermal images [11-13] found the close relation of the orbital area and the base of the ear surface temperature with the rectal temperature. Detection of laminitis in cattle has also been used to screen the hoof health of farm animals [14]. The measurement of stress and the time of establishing social hierarchy in weaned piglets were studied using infrared thermography [15]. The different parts of the body surface reported a significant correlation with salivary cortisol and heart rate in response to handling and transportation [16]. Infrared thermal images have also been used for assessing the early detection of mastitis in cattle [17]. Yadav et al., [18] reported the correlation of thigh temperature with rectal temperature in heat-stress crossbred cattle. They measured the surface temperature of different anatomical sites of heat stress animals using infrared thermal images and suggested that the measurement of thigh skin temperature might be useful as an alternative to rectal temperature in heat stress cattle.

Conclusion

The analysis of surface temperature and its correlation with the core body temperature, reproductive health, disease, thermal stress, and distress in male and female animals for suitable intervention to minimize the stress on animals in pre-clinical studies is necessary for the betterment of the experimental outcome. Surface temperature varies with breeds, species, and responses to different stimuli. Therefore, the generation of baseline data and validation of the surface temperature of different anatomical sites as a representative of rectal temperature, physiological stages, and health status considering the micro-environmental condition of the experimental animals provide a probable application of infrared thermal images (non-invasive, contactless) for selection of experimental animals to avoid the testing on stressed animals.

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