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Utility of facial thermography in the recognition of acute pain in hospitalized cats (*Felis catus*)

Edwin Fernando Buriticá Gaviria[1,2], Paula Alexandra Buitrago-Urueña[1], Maria Camila Torres Pinilla[1], Diego Fernando Echeverry Bonilla[1], Mariângela Endrighi[2], Bruna Natali da Costa[2], Marlos Gonçalves Sousa[2]*

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[1]*Centro Universitario Hospital Veterinario Bernardino Rodríguez Urrea, Universidad del Tolima, Santa Helena parte alta. Ibagué, TOL, Colombia. E-mail: efburiticag@ut.edu.co / ORCID 0000-0002-7979-4135.*
[1]*Centro Universitario Hospital Veterinario Bernardino Rodríguez Urrea, Universidad del Tolima, Santa Helena parte alta. Ibagué, TOL, Colombia. E-mail: pabuitragou@ut.edu.co, ORCID 0009-0004-1854-5671.*
[1]*Centro Universitario Hospital Veterinario Bernardino Rodríguez Urrea, Universidad del Tolima, Santa Helena parte alta. Ibagué, TOL, Colombia. E-mail: mariactorres25@gmail.com, ORCID 0009-0009-3457-951X.*
[1]*Centro Universitario Hospital Veterinario Bernardino Rodríguez Urrea, Universidad del Tolima, Santa Helena parte alta. Ibagué, TOL, Colombia. E-mail: decheverry@ut.edu.co, ORCID 0000-0003-2005-5116.*
[2]*Laboratorio de cardiologia comparada, Universidade Federal do Paraná, R: dos Funcionarios, 1540. CEP 80.035-050. Curitiba, PR, Brasil. E-mail: efburiticag@ut.edu.co, ORCID 0000-0002-7979-4135.*
[2]*Laboratorio de cardiologia comparada, Universidade Federal do Paraná, R: dos Funcionarios, 1540. CEP 80.035-050. Curitiba, PR, Brasil. E-mail: mari.endrighi@gmail.com, ORCID: 0000-0002-0591-7649.*
[2]*Laboratorio de cardiologia comparada, Universidade Federal do Paraná, R: dos Funcionarios, 1540. CEP 80.035-050. Curitiba, PR, Brasil. E-mail: mvbrunacosta@gmail.com, ORCID: 0000-0002-4813-7853.*
[2]*Laboratorio de cardiologia comparada, Universidade Federal do Paraná, R: dos Funcionarios, 1540. CEP 80.035-050. Curitiba, PR, Brasil. E-mail: marlos98@ufpr.br, ORCID: 0000-0003-1367-9828.*

*Corresponding Author: E-mail: efburiticag@ut.edu.co

Abstract: Different clinical conditions have been associated with pain in animals. So, understanding the pathophysiological changes associated with their response is essential in in-hospital clinical monitoring. Facial thermography is a safe and non-invasive diagnostic method that complements other methods of identifying cat pain. This study aimed to describe the usefulness of facial thermography in the recognition of acute pain in hospitalized cats (*Felis catus*). 71 cats were studied with an average weight of 4.3 (±1.4) Kg and an average of 4.4 (±3.5) years old. Facial temperature values were obtained in different region of interest (ROI) and compared according to the values of the Grimace pain scale in cats according to the following subclassification: Group 1, control animals (n=10); Group 2, animals classified with score 0-3 (n=21); Group 3, animals classified with score 4-6 (n=30) and Group 4, animals classified with score 7-10 (n=10). The global average temperature of the facial (F), auricular edge (A), periocular (PO), nasal (N), and rectal (R) regions was 33.7, 31.4, 32.1, 28.9 and 38.3 ºC respectively. A statistically significant difference ($p<0.05$) was observed between the temperature of A and PO and between PO and N, but not between A and N in G4 animals compared to other groups. The present study established a correlation between temperature R and the maximum temperature point in the PO region across all groups (R^2=0.6789, 0.6400, 0.8060, and 0.8126 for groups G1, G2, G3, and G4, respectively). It also revealed a statistically significant increase ($p = 0.0484$) in the mean PO ROI temperature in G4 animals compared to G1, suggesting increased temperature in the lacrimal caruncle associated with pain. Additionally, a statistically significant increase in the mean temperature in the PO mv region was associated with severe pain, with an odds ratio of 6.214 ($p = 0.0168$). It is concluded that the increase in temperature of the periocular region and the nasal plane observed through infrared thermal imaging of the facial surface of hospitalized cats suggests the presence of clinically relevant acute pain.
Keywords: feline; nociception; stress; temperature; thermal window.

1. Introduction

Pain has been recognized and defined, according to the International Society for the Study of Pain (IASP) as an unpleasant sensory and emotional experience similar to potential tissue injury (IASP., 1979; Pérez, 2020). This is a phenomenon that can lead to various physical and behavioral alterations, including an increase in physiological constants, modification of parasympathetic tone, aggression, isolation, or sleep disturbance, among others (Hernández et al., 2019) conditions that over time lead to a deterioration in the quality of life (Reid et al., 2018). Although in recent years diagnostic tools have emerged that allow for a better approach to the clinical identification of pain, such as the use of microneurography, electroencephalography, positron emission tomography, functional magnetic resonance imaging (Mogil, 2022) or the parasympathetic tone activity index with the complementary analysis of various thermographic images (Ghezzi et al., 2024), it is a fact that today the identification of pain in cats (*Felis catus*) is often overlooked due to its limited recognition, subjectivity, and variability in the results of the different methods used to identify pain in this specie.

In hospital practice, acute pain arises from traumatic events, surgical procedures, medical interventions, or infectious processes in both humans and animals (Williams, 2019). In many cases, the onset of pain is abrupt, and its duration is related to the severity of the trigger (Monteiro et al., 2023). These conditions are traditionally associated with behavioral changes; however, the feline species exhibit specific behaviors that can complicate the identification of pain. Some cats alter their behavior and conceal typical manifestations of pain response, such as vocalization (Nibblett et al., 2015).

On the other hand, the use of medications such as tranquilizing drugs, along with various conditions that are common in hospital practice, such as anxiety, stress or fear, can lead to biases in the interpretation of the pain state by affecting the values of physiological, biochemical, or behavioral constants (Steagall and Monteiro, 2019; Steagall et al., 2022); Similarly, the use of scale-based pain assessment methodologies, such as the Colorado State University Feline Acute Pain Scale (CSU-FAPS) or the Glasgow Composite Measure Pain Scale (CMPS-Feline) (Gruen et al., 2022), it is clear that they require the examiner to approach or handle the animal, which could induce changes in the animal's behavior in the intrahospital setting. Therefore, the availability of various diagnostic tools is necessary to characterize pain in different clinical contexts. In this regard, a pain identification scale based on facial expressions, called the Grimace scale in reference to the facial expression produced by the presence of nociceptive stimuli, is widely used in the identification of pain in experimental animals (Domínguez et al., 2023), as well as in different domestic species such as horses, cows, pigs, cats, and even humans (Mogil et al., 2020). It has advantages over those previously mentioned since it does not require palpating the animal and has been validated (Evangelista et al., 2019). For this reason, it has been used as a screening test to identify pain in cats in this study.

Thermographic cameras allow for the identification of changes in blood flow and associate these changes with a color image based on temperature variations obtained at different measurement points. This is because thermographic cameras detect infrared radiation emitted by the surface of the skin and convert it into electrical signals (Kesztyüs et al., 2023). These cameras have margins of error < 0.1°C (± 2%). They use short measurement times (< 0.25 seconds) and can be employed without exposing the animal to unnecessary stress (Sellier et al., 2014), characteristics that make them a versatile and safe diagnostic support tool in clinical practice. In clinical practice, temperature measurement through infrared thermal imaging has been used in different clinical contexts, both in humans (Nahm, 2013) and in animals (Pouzot et al., 2018; Casas et al., 2022). In that sense, the primary use has been focused on detecting changes in tissue circulatory patterns by increasing body temperature or the temperature of a specific body region.

The study of thermographic changes has been studied in various contexts, such as early diagnosis and prognosis in orthopedic or soft tissue injuries in horses (Eddy et al., 2001), detection and evaluation of the estrous cycle in sheep (Barros de Freitas et al., 2018), cancer in animals (Holanda et al., 2023), and in humans with intraocular tumors and breast cancer (Travain et al., 2015; McManus et al., 2016; Modrzejewska et al., 2021). Moreover, it has been used as a predictor of clinical alterations, even before any clinical signs appear, to prevent the spread of infection (Rainwater-Lovett et al., 2009; Grossbard et al., 2014), assessment of autonomic nervous system activity (Ghezzi et al., 2024), monitoring peripheral vascular disorders (Mota et al., 2023), diagnosing vaccination-induced fibrosarcoma (Nitrini et al., 2021), and accompanying the monitoring of body temperature (Giannetto et al., 2021).

An association between pain and skin temperature, measured by thermography, has been demonstrated in humans (Zhang et al., 1999; Nahm, 2013) and animals (Casas et al., 2020; Travain and Valsecchi, 2021) repeated. This association is attributed to the vascular behavior on the skin surface. In cats, thermography has been primarily utilized for monitoring aortic thromboembolism (Pouzot et al., 2018); despite these findings, there are currently no established facial thermographic ranges in felines to utilize this method for diagnosing the clinical state of pain. The objective of the present study was to determine the usefulness of facial thermography images in recognizing acute pain in hospitalized cats (*Felis catus*).

2. Materials and Methods

2.1. Animals

A total of 71 cats from the following breeds were included: ordinary domestic shorthair (n=61), Siamese (n=6), and Bengal (n=4), with an average weight of 4.3 (±1.4) kg and an average age of 4.4 (±3.5) years. The animals included in this study were admitted to the hospitalization service of the Centro Universitario Hospital Veterinario Bernardino Rodriguez Urrea of the Universidad del Tolima (Colombia). for various reasons, categorized as follows: trauma (n=16), gastroenteritis (n=11), cystitis or urinary tract obstruction (n=11), cancer (n=7), respiratory failure (n=5), cholecystitis (n=3), seizures (n=2), pyometra or other reproductive disorders (n=2), gingivostomatitis (n=2), glaucoma (n=1) and poisoning (n=1). The control group (n=10) consisted of clinically healthy animals brought to the institution for routine clinical check-ups.

2.2. Thermographic image capture

All animals underwent a minimum 10-minute acclimatization period in the hospital cage, ensuring no evidence of natural or artificial air currents that could impact image capture. Thermographic images of the facial region were captured in all cats using a thermal imaging camera (FLIR e50, Flir Systems, Inc., Wilsonville, USA). Recordings were taken at a distance of 1.0 m, focusing on each individual's nose. Emissivity was adjusted to 0.98 in all cases.

Subsequently, various anatomical reference points were analyzed utilizing the specific software of the thermographic device (Teledyne FLIR Thermal Studio, Flir Systems, Inc., Wilsonville, USA) (Figure 1). These were defined as anatomical reference points for this study: the global facial region (F), the auricular edge (A), the periocular region (PO), and the nasal region (N). Each of the anatomical points evaluated was individually recognized in this study as a region of interest (ROI). The average temperature in each ROI was obtained from three measurements, and the resulting value was chosen for statistical analysis. Likewise, the maximum and minimum points of each ROI were identified as F, A, PO, and N according to a 4-quadrant thermographic distribution pattern. This pattern included the following regions: the right dorsolateral region, the left dorsolateral region, the right ventrolateral region, and the left ventrolateral region for sample N. Additionally, for regions A and PO, the points were identified in the dorsolateral, dorsomedial, ventrolateral, and ventromedial regions.

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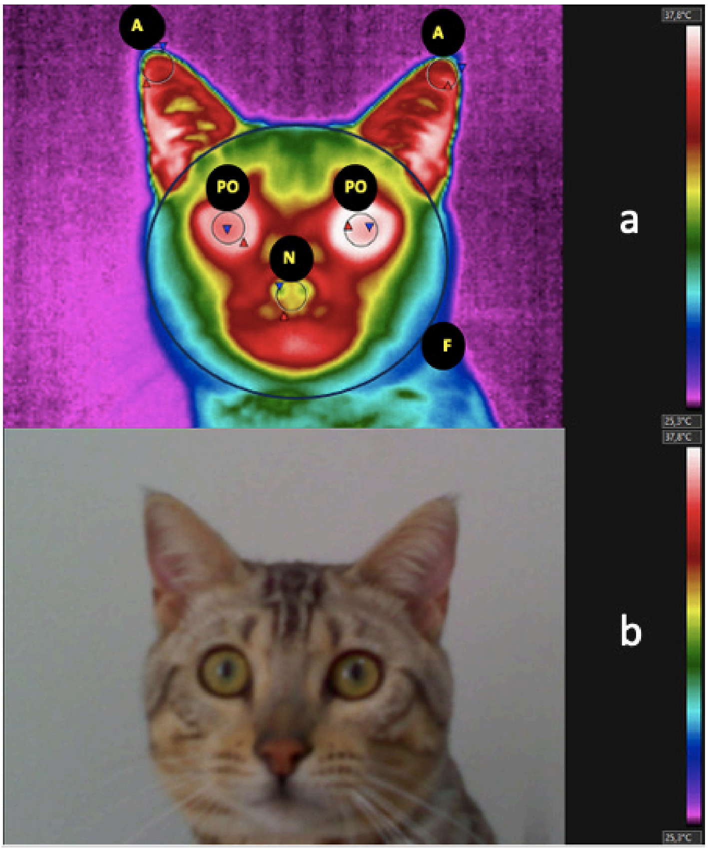

Figure 1 – Thermographic image (a) and regions of interest (ROI) for the different facial temperature measurements in cats obtained in the study: global facial region (F), auricular edge (A), periocular region (PO), and nasal region (N). The maximum and minimum temperature points of each point are represented by the red and blue arrows, respectively. (b) A standard color picture of the same animal.

2.3. Intraobserver and interobserver reliability

Fifteen images recorded in the study were randomly chosen. The same evaluator analyzed the images again 30 days later and obtained an average of the temperature from three consecutive measurements in each of the ROIs to calculate the intra-observer variability. Using the same images, another evaluator performed the same measurements without knowledge of the previous results, aiming to estimate interobserver variability. All measurements were analyzed utilizing the specific software of the thermographic device (Teledyne FLIR Thermal Studio, Flir Systems, Inc., Wilsonville, USA).

2.4. Experimental design

The hospitalized animals were subclassified into four groups based on the Grimace scale scores obtained as follows: Group 1, control animals (n=10); Group 2, animals classified with a score of 0-3 (n=21); Group 3, animals classified with a score of 4-6 (n=30); and Group 4, animals classified with a score of 7-10 (n=10). Groups 2, 3, and 4 were associated with mild, moderate, and severe pain levels, respectively.

The temperature of each anatomical point was individually analyzed and compared with the contralateral region. The average temperature value between each anatomical point was also compared. Similarly, differential gradients were established according to rectal temperature.

Cats of long-haired breeds, those with brachiocephalic conformation, facial anatomical alterations, in a state of sedation, or aged less than four months were excluded from the study (n = 5).

2.5. Statistical analysis

All data were tabulated and analyzed using descriptive statistics, determining minimum and maximum values and average and standard deviation variables. The normal distribution of the data was assessed using the Shapiro-Wilk test. Group comparisons were conducted through the one-way analysis of variance (ANOVA) test, with Tukey's test employed as a post-hoc analysis. Non-parametric variables were compared using the Kruskal-Wallis test. A significance level of $P<0.05$ was set. The relationship between variables was performed using Pearson's correlation coefficient or Spearman's Rho as appropriate, and consequently, the coefficient of determination (R^2) was estimated. The agreement between blind observers was evaluated using the intraclass correlation coefficient (95% CI) for each measurement point. The intraclass correlation values were defined as follows: very strong (0.81–1.00), strong (0.61–0.80), moderate (0.41–0.60), weak (0.21–0.40), and very weak (<0.20). Finally, odds ratios (OR) and 95% confidence intervals (95% CI) were obtained using Fisher's exact test. All statistical analyses and graphs were generated using GraphPad Prism v 8.0 (La Jolla, USA) and BioEstat v 5.3 of the Instituto de Desenvolvimento Sustentável Mamirauá (Belém, Brazil).

3. Results

The global average temperatures of the facial (F), auricular edge (A), periocular (PO), nasal (N), and rectal (R) regions were 33.7, 31.4, 36.1, 28.9, and 38.3 °C, respectively. A statistically significant difference ($p<0.05$) was observed between the temperatures of A and PO and between PO and N, but not between A and N.

Only a statistically significant difference ($p=0.0484$) was observed in the PO of G1 compared to G4 (Figure 2). Table 1 presents the average values and their standard deviation (±SD) of the temperature in each ROI for each evaluated group.

A weak correlation ($R^2=0.2357$) was observed between the R and the PO temperature. However, when evaluating the correlation between the R and the maximum temperature point in the PO region, consistently located in the middle ventral quadrant in all cases, the correlation was: $R^2=0.6789$, 0.6400, 0.8060, and 0.8126 for groups G1, G2, G3, and G4, respectively. The correlation between temperature and the age or weight of the cats evaluated was $R^2=0.1501$ and $R^2=0.1201$ respectively.

A correlation observed between the Grimace scale values and the temperature in each of the ROI's F, A, PO and N was $R^2=0.1312$, 0.1220, 0.1280, and 0.1090 respectively. The highest correlation between the Grimace scale and an ROI was observed in the POvm region ($R^2=0.5956$).

Parameters (ºC)	G1 (n=10)	G2 (n=21)	G3 (n=30)	G4 (n=10)	P value
Facial (F)	32.6 (±2.1)	34.0 (±1.6)	33.5 (±1.7)	34.6 (±2.2)	0.0828
Auricular edge (A)	29.9 (±4.5)	32.0 (±3.0)	31.2 (±3.3)	32.0 (±3.5)	0.6712
Periocular (PO)	35.6 (±0.9)	36.2 (±0.5)	36.0 (±0.8)	36.6 (±1.2)*	0.0484
Nasal (N)	26.4 (±3.2)	29.3 (±4.5)	28.5 (±4.3)	31.2 (±5.2)	0.0607
Rectal (R)	38.2 (±0.3)	38.3 (±0.8)	38.4 (±1.0)	38.4 (±0.6)	0.7080
Difference R-F	7.6 (±2.9)	5.8 (±2.2)	6.5 (±2.7)	5.2 (±3.0)	0.1719
Difference R-A	8.4 (±5.3)	6.3 (±2.7)	7.2 (±3.5)	6.5 (±3.4)	0.7794
Difference R-PO	2.7 (±1.0)	4.7 (±3.5)	4.2 (±2.6)	3.7 (±2.5)	0.6698
Difference R-N	11.9 (±3.2)	9.0 (±4.5)	9.9 (±4.4)	7.2 (±5.1)	0.1111
Difference N-A	2.7 (±3.0)	3.5 (±2.9)	3.1 (±2.7)	2.8 (±1.4)	0.7256
Difference PO-A	5.7 (±4.6)	4.1 (±2.6)	4.8 (±2.9)	4.5 (±2.6)	0.7021

Table 1 – Mean and standard deviation (±S.D.) of facial (F), auricular edge (A), periocular (PO), nasal (N), and rectal (R) temperatures (°C) in cats subclassified according to the Grimace Facial Expression Pain Scale score. Control cats (G1), Grimace scale 0-3 (G2), Grimace scale 4-6 (G3), and Grimace scale 7-10 (G4).

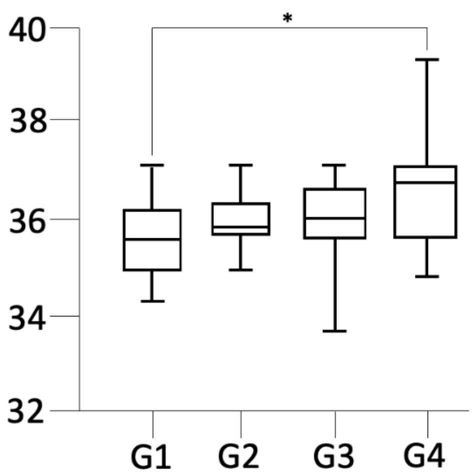

Figure 2 – Box plot showing mean (± SD) values of periocular temperature in cats subclassified according to the Grimace Facial Expression Pain Scale. Control cats (G1), Grimace scale 0-3 (G2), Grimace scale 4-6 (G3), and Grimace scale 7-10 (G4). *Statistical difference $p < 0.05$.

The subjective thermographic analysis demonstrated a pattern in the control group animals characterized by the formation of a V-shaped image, starting from the ventromedial (mandibular) region in a dorsolateral direction (auricular edge), with greater thermal intensity (hot) in the palpebral edges and lesser (cold) in the area of the nose. Animals from G3 and G4 demonstrated an increase in the thermogram on the dorsal surface of the nasal plane towards the PO region (Figure 3). Similarly, the location subjective of points of the maximum and minimum temperature recorded in the sample areas A and PO showed a homogeneous distribution pattern in 68/71 (95.8%) and 53/71 (74.6%) of the animals, respectively. Of these, only the maximum temperature recorded in the PO region was statistically higher ($p=0.0464$) in G4 compared to G1. The maximum and minimum temperature points recorded in samples F and N showed no defined pattern in more than 50% of the cases.

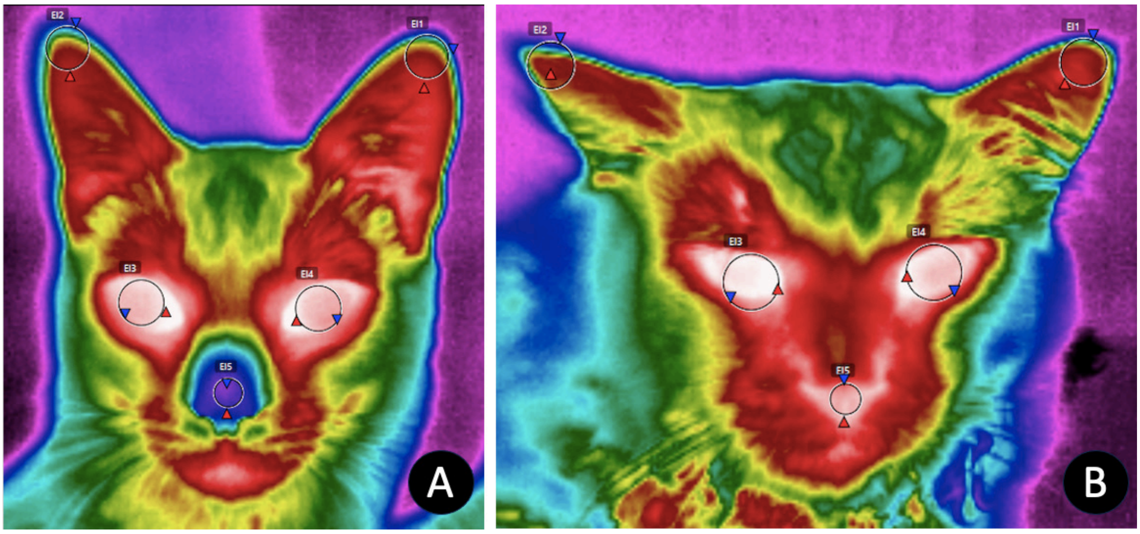

Figure 3 – Differences in the thermographic images of two cats according to the Grimace Facial Expression Pain Scale score. Image (A) corresponds to a cat from the control group G1, while image (B) corresponds to a cat with a Grimace scale 7-10 from G4. The red arrows indicate the hottest point of measurement, and the blue arrow indicates the coldest. The circles correspond to the regions of interest (ROIs) in the thermographic sample.

The agreement between observers was vital for the variables A, PO, and N, with intraclass correlations of 0.85, 0.90, and 0.95, respectively, but moderate for the variable F (0.54). The Bias analysis was non-significant for all the variables, with PO having the lowest value (Table 2 and Figure 4).

Variable	Bias (ºC)	SD (ºC)	LOA	CCI
Intraobserver				
Facial (F)	0.54	1.86	-3.10 to 4.18	0.54
Auricular edge (A)	0.55	1.70	-2.78 to 3.89	0.85
Periocular (PO)	0.12	0.46	-1.03 to 0.79	0.90
Nasal (N)	0.59	1.21	-1.79 to 2.96	0.95
Interobserver				
Facial (F)	0.60	1.18	-1.66 to 2.98	0.71
Auricular edge (A)	0.53	1.47	-2.36 to 3.41	0.87
Periocular (PO)	0.19	0.45	-1.06 to 0.69	0.90
Nasal (N)	0.66	1.18	-1.66 to 2.98	0.95

Table 2 – Bland-Altman bias, standard deviation (±SD), limits of agreement (LOA), and intraclass correlation coefficient (ICC) of facial temperature (°C) in cats across various regions of interest for intraobserver and interobserver analyses.

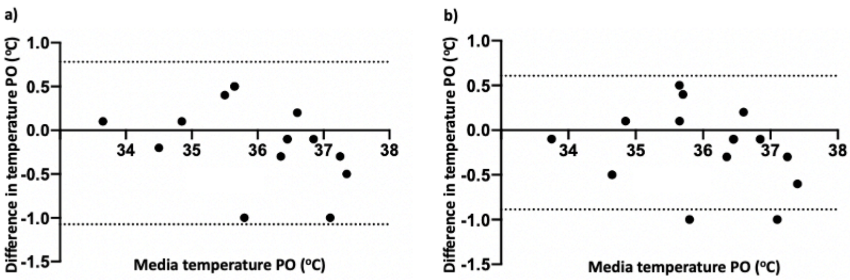

Figure 4 – Bland-Altman graphic representing intra (a) and inter-observer (b) agreement for the media periocular temperature values (PO) in degrees Celsius (ºC).

According to the Grimace scale, the most prevalent pathologies associated with moderate and severe pain were trauma (11/61, 18.0%), gastroenteritis (6/61, 9.8%), cystitis or urinary tract obstruction (6/61, 9.8%), and cancer (5/61, 8.2%). Frequency distributions concerning the diagnoses of hospitalized cats are contained in Table 3, and the OR for the main diagnoses is found in Table 4.

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Diagnostic	n	G2 (n=21)	G3 (n=30)	G4 (n=10)
Trauma	16	5/61(8.2%)	5/61(8.2%)	6/61(9.8%)
Gastroenteritis	11	5/61(8.2%)	5/61(8.2%)	1/61(1.6%)
Cystitis or Urinary tract obstruction	11	5/61(8.2%)	5/61(8.2%)	1/61(1.6%)
Cancer	7	2/61(3.3%)	4/61(6.6%)	1/61(1.6%)
Respiratory failure	5	1/61(1.6%)	3/61(4.9%)	1/61(1.6%)
Cholecystitis	3	2/61(3.3%)	1/61(1.6%)	-
Seizures	2	-	2/61(3.3%)	-
Pyometra or other reproductive disorders	2	1/61(1.6%)	1/61(1.6%)	-
Gingivostomatitis	2	-	2/61(3.3%)	-
Glaucoma	1	-	1/61(1.6%)	-
Poisoning	1	-	1/61(1.6%)	-

Table 3 – Number of animals (n) and percentage distribution (%) of each diagnostic group in each subgroup according to the Grimace Facial Expression Pain Scale score. Grimace scale 0-3 (G2), Grimace scale 4-6 (G3), and Grimace scale 7-10 (G4).

Four animals in the study presented increased tear production with evident epiphora (gingivitis in 2, glaucoma in 1, head trauma in 1) without statistically significant differences between them and the control group (p=0.2231).

Diagnostic	OR	95% CI
Trauma	0.2014	0.059 to 0.692
Gastroenteritis	1.2240	0.413 to 5.387
Cystitis or Urinary tract obstruction	1.0220	0.297 to 3.548
Cancer	1.7140	0.833 to 71.07

Table 4 – Odds ratios (OR) and 95% confidence intervals (95% CI) for the four diagnoses included in the study were calculated at a temperature discriminator of 36.1°C in the PO region.

OR and 95% CI for the increase in the average temperature of each ROI and the presence of severe pain were as follows: 0.372, 0.6432 to 7.953; 0.9429, 0.2748 to 3.280; 1.615, 0.4128 to 5.387 and 1.657, 0.4754 to 5.756 (p > 0.05) for regions F, A, PO, N respectively. Finally, the OR and 95% CI for the PO ventromedial region (POvm) and PO ventrolateral region (POvl) were 6.214, 1.299 to 30.180 (p=0.0168) and 3.913, 0.8384 to 20.030 (p=0.1351) respectively.

4. Discussion

The present study demonstrated a statistically significant increase (p=0.0484) in the average temperature of the PO region in G4 animals compared to G1. It also showed that an increase in temperature in the POmv region was associated with an OR of 6.214. To the authors' knowledge, this is the first study worldwide to illustrate facial thermographic behavior in hospitalized cats with different degrees of acute pain according to the Grimace classification.

In our study, the maximum temperature of the PO region was consistently located at the ventromedial point of the periocular sample in 68/71 (95.8%) of the animals, compared to other facial points. The facial thermogram in cats, and mainly in dogs, indicates an increase in temperature in the region of the lacrimal caruncle, an anatomical area close to the internal palpebral angle. Due to its vascularization, this region responds with a thermal increase attributed to the activation of the autonomic nervous system in response to stress (Foster and Ijichi, 2017; Casas et al., 2022). The thermal points of the PO region of the cats had the highest recorded temperature values in the facial thermographic records, suggesting an anatomical reference point in this analysis profile. These findings were subjectively correlated with increased hot thermographic density in an area between the eyeball and the nose, potentially corresponding to the described region in the present study. Temperature increases in the PO region suggest have been linked to stress in cats (Foster and Ijichi, 2017) and other species such as rats, rabbits, pigs and even humans in a phenomenon known as stress-induced hyperthermia (Nakamura, 2015; Travain and Valsecchi, 2021) partly due to the autonomous innervation of the capillaries in the tear canaliculi (Mota et al., 2022a). Furthermore, the thermographic responses observed in the PO region, along with facial expressions, are likely to aid in determining the degree of pain (Antonaci et al., 2019). This evidence was also observed in our study, where the position of whiskers, eyelid opening, and ear positioning were associated with severe pain in all animals in G4.

In this present study, a weak but statistically significant correlation was observed between R temperature and PO temperature (R^2=0.2357), contrasting with findings by other authors who indicated a stronger positive correlation between ocular temperature and rectal temperature (R^2=0.9300), with an average difference of 1.19 °C (Giannetto et al., 2021). However, a strong positive correlation (R^2=0.7204) was evident in our study when analyzing the highest regional temperature point in the PO region, consistently located in the middle ventral quadrant in all cases. This correlation was higher in G4 (R^2=08126), suggesting that thermal changes associated with pain stress are linked to increased temperature in this anatomical region close to the lacrimal caruncle. This finding aligns with previous reports (Biondi et al., 2015; Mota et al., 2022a) highlighting the lacrimal caruncle as the hottest part of a cat's facial thermogram. Our study considers this region a strategic anatomical site for thermographic monitoring of cats with pain, in agreement with authors associating these changes with acute responses secondary to the release of

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proinflammatory substances, leading to vasodilation and increased energy radiation emitted by the animal (Mota et al., 2022b). It's important to note that different tear film alterations could impact infrared thermography measurements, potentially influencing the global eye temperature (Biondi et al., 2015) and affecting its agreement with rectal temperature. While no observations related to tear film quality were made in this study, none of the animals showed clinically relevant ocular alterations. Therefore, we assume the observed changes were directly related to the stress scale associated with individual patient's pain.

The clinical conditions that scored highest on the Grimace scale and exhibited the highest maximum temperature values in the PO and N regions were trauma, gastroenteritis, cystitis or urinary tract obstruction, and cancer. Various clinical conditions frequently encountered in hospital practice and associated with different types of pain include musculoskeletal disorders caused by trauma or degenerative disorders, obstructive and non-obstructive urogenital disorders, dental disorders, eye injuries, and even different routine hospital procedures (Hellyer et al., 2007). The most prevalent clinical conditions found in this study are associated with moderate to severe levels of pain (Monteiro et al., 2023); however, in our study, the diagnoses observed were not strongly correlated with the overall values of the different ROIs except for the POvm region (R^2=0.5956). This study enabled us to demonstrate that increases in the mean temperature of the POvm region are associated with an OR of 6.214 regarding the presence of severe pain compared to those without increases in mean temperature at that anatomical point. We suggest that the elevation of temperature at this thermographic point is influenced by the anatomical region of the lacrimal caruncle and, therefore, has a more sensitive vascular response to the changes associated with pain in cats. This could be primarily attributed to the inflammatory events accompanying these ailments. However, it is essential to acknowledge that animals in pain may simultaneously exhibit behavioral changes associated with fear, anxiety, and stress when in a hospital environment (Steagall et al., 2022), thus potentially influencing the observed thermographic changes in this study.

In this study, all animals were assessed in controlled in-hospital conditions, and measures were implemented to minimize artifacts. Various factors can influence thermographic images, including the incidence of solar rays, humidity, or air currents on the surface being evaluated (Vainionpää, 2014). While the minimum presence of artifacts is assumed, some clinical conditions like gingivitis, eye disease, or head trauma may have affected recordings. In our study, four animals (2 with gingivitis, 1 with glaucoma, and 1 with head trauma) presented mild epiphora; however, no statistically significant differences were observed between this group of animals and those in the control group (p=0.2231), leading us to assume that such findings do not influence the results. Despite this, it's important to note that inflammatory processes have been linked to increased surface temperature after using thermographic images (Vianna and Carrive, 2005). However, the presence of moisture, as in this case the accumulation of tear fluid in the periocular region, could at least partially underestimate the value of the temperature in this ROI (Kwon and Brundage, 2019; Mota et al., 2022a).

This study found strong agreement values for intra-rater reliability at all measurement points. However, measurements at the auricular point exhibited variability compared to blind observers, with a tendency to be underestimated. Meanwhile, inter-rater reliability ranged from strong to low bias, particularly for the PO region. This phenomenon may be explained by the ROI size chosen by each evaluator during post-analysis. A smaller ROI size could decrease overall temperature measurements at each sample point, as the temperature of the eyelid margins is consistently higher than that of the corneal surface (Mota et al., 2022a). Despite these variations, the findings suggest that intra- and inter-observer analyses were within an acceptable range of correlation, especially for the temperature measurements of the N and PO regions. This implies that cat facial thermographic evaluation could be a reliable and reproducible technique for diagnosing acute pain.

Finally, the quality of the thermographic device can impact the images obtained, as the camera resolution can influence variation between measurements in different thermographic studies. In biomedical applications for humans, the most frequently encountered resolution is 320x240 pixels (76.800 temperature points) (Kesztyüs et al., 2023). In our study, images were captured with a camera boasting a resolution of 240x180 pixels (43.200 temperature points), providing adequate resolution for biomedical image analysis, exceeding at least 19.200 temperature points per image frame as proposed in the guidelines set by the International Academy of Clinical Thermology for quality assurance in humans (Amalu, 2018). Higher resolution contributes to better repeatability; therefore, we consider that the use of thermographs with a resolution greater than 180x180 pixels (32.400 temperature points) is deemed most appropriate for veterinary biomedical analysis (Vainionpää, 2014). Kesztyüs et al. (2023) analyzed the characteristics of different thermal imaging cameras used in human medicine and described FLIR brand devices as the most frequently referenced in obtaining thermographic images for biomedical use; however, this analysis has not yet been carried out in veterinary medicine. We consider that the images obtained with the FLIR e50 camera meet the minimum requirement necessary to obtain differential images for pain monitoring in cats using facial thermography, but we cannot affirm that a slightly lower resolution would allow obtaining the same results.

Despite efforts to ensure a homogeneous environmental temperature, a limitation was encountered regarding the capture of thermographic images at different times of the day, depending on the hospital admission time of each specimen. This situation could lead to variability in the thermographic records obtained in the study, as variations in environmental temperature throughout the day may have occurred, which should have been considered by the researchers. Additionally, no records of environmental temperature were obtained, preventing the identification of potential associations. In this study, the presence of pain was determined solely based on the Grimace scale score, without consideration of other scales or physiological parameters for interpretation. It is suggested that future studies take these considerations into account.

5. Conclusion

The observed increase in temperature in the ventromedial periocular region and the nasal plane, as captured through infrared thermal imaging of the facial surface in hospitalized cats, may suggest the presence of clinically relevant acute pain. Facial thermography proves to be a safe and non-invasive diagnostic method that can complement other approaches to identifying cat pain. Nevertheless, further studies are needed to fully understand its utility in recognizing pain across various hospital clinical contexts.

Conflict of Interest: The authors declare no potential conflicts of interest regarding this article's research, authorship, and publication.

Declarations and Ethics: This study received approval from the Bioethics Committee of the University of Tolima and adhered to the guidelines outlined in Laws 84 of 1989 and 1774 of 2016 concerning animal protection in Colombia.

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