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"Agreement study between esophageal and cloacal thermometry or infrared thermography measurements in Eurasian eagle-owl (*Bubo bubo*)"

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ABSTRACT

Body temperature is a marker of health or disease in wild birds. In avian species, the most common clinical method for determining body temperature is cloacal thermometry; however, there is little information on how it represents central body temperature in most avian species, including the Eurasian eagle-owl. Also, a less invasive method, such as infrared thermography, may reduce stress during clinical procedures. This study aims to perform a concordance analysis between cloacal thermometry and ocular or pedes thermographic measurements taken from Eurasian eagle owls, comparing them to esophageal thermometry, which is considered a good gold standard for its anatomical arrangement. The study was performed during the clinical procedures carried out on owls at the recovery center hospital. Thermometric measurements (esophageal, cloacal) and thermographic images of the pedes and eyes were documented. The highest correlation was observed when esophageal thermometry values were compared to cloacal thermometry values or eye thermography. The obtained agreement values showed a bias (upper limit of agreement (LoA_u)- lower limit of agreement (LoA_l)) of −0.30(0.90 to −0.76), 0.20 (2.77 to −0.90), 0.30(2.77 to −0.86), and 0.30(3.62 to −4.16) °C, when esophageal thermometry was compared to cloacal thermometry or thermography values obtained from entire eye, medial canthus of the eye, and pedes, respectively. The cloacal temperature had better correlation and agreement with esophageal thermometry than the thermographically studied sites. The ocular temperature showed a closer agreement with esophageal temperature than with the temperature measured at the pedes. Neither cloacal nor thermographic temperature measurements are concordant with esophageal temperature values (>0.5 °C).

1. Introduction

Body temperature is one of the most relevant clinical signs used as a marker of health or disease. It is important in avian medicine because it offers essential insights that aid clinicians in decision-making (Liles and Di Girolamo, 2023). Additionally, examining animal body temperature is necessary from physiological, ecological, and evolutionary points of view (Gauchet et al., 2022). Also, it may be a tool to assess the animal's stress level, which is particularly important in wild birds (McCafferty et al., 2015). Although thermography may serve as a valuable tool for assessing acute stress in natural environments, given the relationship between surface temperature and peripheral vasomotor activity, the specific aspects of stress physiology with which it correlates remain unclear. (Jerem and Romero, 2023). It has been reported that this tool could provide a useful noninvasive measure of the activity of hypothalamic-pituitary-adrenal axes (HPA) in acute stress, however it might not be suitable for measuring glucocorticoids levels (Ouyang et al., 2021). Also, the importance of the environment or individual context such as social hierarchy in shaping physiological responses to stressors should be considered (Robertson et al., 2020a, 2020b). Healthy

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homeothermic animals can maintain their body temperature within intrinsic ranges for each species through thermoregulatory mechanisms (Giannetto et al., 2021; Ruuskanen et al., 2021). Therefore, thermal disruption not attributable to extreme external conditions or food ingestion is associated with underlying pathological conditions (Perry et al., 2020; Liles and Di Girolamo, 2023). These reasons highlight the importance of determining the physiological temperature ranges in different species and searching for the best tool and site for measuring this physiological parameter.

Central body temperature reflects the temperature of internal organs, and intravascular temperature measurement is the gold standard (Cutuli et al., 2021). However, the complexity and risks associated with this measurement have positioned cloacal temperature as one of the most common practices in avian species daily clinical care (Kreissl and Neiger, 2015), and it serves as an indicator of central temperature (McCafferty et al., 2015; Cremer et al., 2019). However, we must not overlook its limitations, such as the delay in response to sudden temperature changes and the likelihood of underestimating central body temperature (Lies et al., 2023). This would not represent a problem as long as the reference range for that area can be established and/or a conversion factor could be applied to these measures (Giannetto et al., 2022). Regarding anesthetic management, the esophagus's proximity to the left atrium and left ventricle, as well as its rapid response to changes in body temperature, have established esophageal temperature as a fundamental location for temperature measurement in anesthetized patients (Hymczak et al., 2021). In mammals, this is a more reliable method for measuring central body temperature than rectal thermometry. Esophageal thermometry is considered the best alternative to arterial thermometry in clinical practice, as it reflects central temperature more closely than other methods (Cutuli et al., 2021).

Cloacal temperature measurement is a widespread practice in avian medicine, but this technique is invasive, time-consuming, and stressful for a wildlife species (Liles and Di Girolamo, 2023; Hernández-Sánchez et al., 2024). Wild birds are particularly prone to fear and stress during clinical procedures. Handling for temperature measurement can exacerbate stress (Doss and Mans, 2017), especially when birds require intensive care with frequent monitoring. A non-invasive thermometric technique, such as thermography, may be an alternative. The broad application potential of this technique is based on its ability to measure any body whose exterior surface temperature is higher than absolute zero. The use of this technique can avoid stress and, therefore, temperature changes induced by handling (Arfuso et al., 2022). However, this method also presents limitations, especially related to the variability of surface temperature depending on environmental factors (McCafferty et al., 2015). Despite its limitations, this technique is very useful in the rehabilitation centers of wildlife birds for assessing the progression of pododermatitis and electrocuted animals by minimizing the necessity for manual restraint (Mota-Rojas et al., 2022). Therefore, we aimed to evaluate the extent to which thermography can provide reliable information on central body temperature, as this would be particularly valuable for brief, routine handling procedures conducted under similar environmental conditions within a facility. This study represents an initial step toward understanding the performance of thermography under controlled conditions. If thermography does not yield clinically relevant information in such settings, its utility for precise temperature monitoring may be limited; however, it could still be valuable for tracking temperature trends.

Despite its importance and the data reported in mammals (Greer et al., 2007; Johson et al., 2011, Nutt et al., 2016; Ruston et al., 2015; Zanghi, 2016; Barbiere et al., 2021; Giannetto et al., 2021; Kim and Cho, 2021; Arfuso et al., 2022), studies on the concordance between thermometric methods in birds are scarce. To our knowledge, there are only two previous studies carried out on red kites and vultures, and only the latter includes thermography. There are significant anatomical differences between the rectum of mammals and the avian cloaca, as well as anatomical differences between avian species (e.g., digestive characteristics with the presence or absence of a crop, different cloaca sizes, ocular characteristics, and different limb lengths and shapes, with or without feathers). These differences could significantly influence the results, which highlights the importance of conducting this type of study on different avian species. This study aims to perform a concordance analysis between cloacal thermometry or thermographic measurements conducted in eye and pedes of Eurasian eagle owls and esophageal thermometry, considered the gold standard. Furthermore, since cloacal temperature is frequently used in agreement studies as a reflection of central temperature, we considered it important to analyze its agreement with thermographic measurements.

2. Material and methods

2.1. Animal and experimental design

After evaluating this protocol, the Ethics Committee of the Complutense University of Madrid, concluded that it does not require approval. A sample of eight Eurasian eagle owls (*Bubo bubo*), of unknown gender and weighing between 1874 and 2496 g, housed at the GREFA. rehabilitation center was used. A prior ophthalmologic and general assessment was conducted, excluding individuals with ocular abnormalities and pododermatitis. The study was performed during the clinical procedures carried out at the recovery center hospital. All animals underwent a 24-h fasting period and were moved to the indoor facility at least 2 h before the study. The owls had at least 15 min of acclimatization to the environmental conditions of the operating room. All recordings were performed between 08:00 and 15:30 h to minimize variations due to the circadian rhythm of body temperature.

The operating room was preheated using an air conditioning unit (Carrier, 42HQV 045-R) to maintain a temperature of 27 °C throughout the procedure. The room temperature was selected within the range usually set at the operating room for these procedures in avian species. The operating table was positioned at 101 cm and 91 cm from the floor at the anterior and posterior ends, respectively, placing the animal in the anti-Trendelenburg position. The surgical lamp (Centurion Excel) was positioned 114 cm from the anterior end of the table, adapting the method established by Carballo et al. (2022). An electric blanket located on the operating table was used to prevent birds' heat loss during the procedures. The blanket was positioned beneath the animal, with its back resting on top of it. Neither the head nor the pedes were on the blanket.

Before anesthesia, owls were placed in dorsal recumbency and oxygenated with a mask (100 %; 2 L/min). Cloacal temperature was measured using a fixed penetration probe thermometer (Cooper Atkins, COO-TM99A-0) to determine the temperature before induction and check if it was within the physiological range (values oscillated between 39.1 and 40.6 °C). Subsequently, the owls were induced, using a face mask, with sevoflurane (8 %; 1.5 L/min) through a calibrated vaporizer (General Anesthetic Services Ltd, Penlon Sigma Elite). Loss of palpebral reflex and muscle tone were used to verify the anesthetic depth before intubation, which was performed with uncuffed endotracheal tubes ranging from 3 to 3.5 mm. Physiological parameters (electrocardiogram waveform, heart rate, capnography, and oxyhemoglobin saturation) were monitored (Digicare LW8xVet, LifeWindowsLite) throughout the study. The cloaca was flushed with 5 ml of tempered (39 °C) saline solution 10–15 min prior to the start of the study. Cloacal and esophageal probes (MED LINKET) were placed, at depths of 3 cm and 22 cm, respectively, following previously established marks based on the center's experience and anatomical validation in cadavers. At least 1 min was allowed for the recorded measurements to stabilize. Anesthesia maintenance was achieved using sevoflurane (3–4.5 %; 1.5 L/min). At the end of the study, sevoflurane delivery was suspended and the anesthetic circuit was washed with 100 % oxygen (1.5 L/min) to allow recovery.

2.2. Measurements

Measurements (esophageal, cloacal, and thermographic) were recorded every minute. During each thermographic sampling cycle, images of the pedes (TGped) and left eye (TGeye) were recorded in that order (Fig. 1). The sampling period varied among the different birds, ranging from a minimum of 13 min to a maximum of 19 min. This resulted in 136 cloacal (Tc) and esophageal (Te) temperature recordings and 272 thermographic images under constant ambient background temperature conditions.

The thermal images were captured using an infrared radiation camera (FLIR Systems, FLIR E75), with a focal plane sensor array size of 320 x 240 pixels. The camera was positioned at 30 cm and 90° angle to the subject, the bird was always placed in the same position to avoid changes in the angle of incidence and, thus, minimize errors. The instantaneous field of view (IFOV), which represents a single pixel into the FOV (24°), was 1.31 mrads. The camera acquired at 30 Hz and a focal length of 17 mm. The temperature range is between −20 and +120 °C, with manufacturer-certified precision of ±0.2 °C and accuracy of ±2 °C or 2 % of reading, respectively. An emissivity of $\varepsilon = 0.95$ was set based on previous studies on avian species (Graveley et al., 2020; Playà-Montmany and Tattersall, 2021).

The thermal images were analyzed using FLIR Tools Software, which allowed for the selection of areas of interest. The entire eye was scanned to obtain the maximum temperature (Tmax) according to Jerem et al. (2015), ensuring that the whole eye of each of the eight Eurasian eagle owls was analyzed. The scanned area was dorsally limited by the upper eyelid margin (limbus palpebralis), ventrally by the lower eyelid (limbus palpebralis), laterally by the lacrimal angle (lateral canthus), and medially by the nasal angle (medial canthus) (Fig. 1A). Additionally, three circle areas with a 7.5-pixel radius were defined to assess thermal differences between the lateral ($TGeye_L$), medial ($TGeye_M$), and central ($TGeye_C$) corneal areas of the eyeball. To define the three internal regions of interest (ROI) within the eye, a reference line was drawn from the medial to the lateral canthus. The circle delimiting the medial ROI was positioned along this line, with its center located 7.5 pixels from the medial canthus. Similarly, the lateral ROI was placed with its midpoint on the same line, 7.5 pixels from the lateral canthus. The central ROI was established by positioning the center of the circle at the midpoint of the reference line (Fig. 1A and Fig. 2A). A scan covering both pedes was performed to locate the point of maximum temperature emission using a circle with a radius of 22 pixels (Fig. 2C). All measurements were performed on the dorsal side and the positions predominantly adopted by the pedes are shown in Fig. 1. The scanned area of the pelvic extremity extends distally from the tarsometatarsus, beginning at the region where the feathers form a slight bulge (Fig. 1D), down to the nails, representing approximately 60 % of the tarsometatarsus length. The feathers were not removed.

The manufacturer's reported accuracy is ±0.1 °C between 25 and 45 °C for cloacal and esophageal probes. Although cloacal and esophageal probes were factory calibrated, thermometers were simultaneously immersed in a thermoregulated water bath between 35 °C and 42 °C, which corresponds to the temperature range expected and observed in our study, and were compared to a mercury thermometer. The maximum difference between the thermometers used in our study with the mercury thermometer was 0.1°C for cloacal and esophageal probes.

2.3. Statistical analysis

A commercial software (IBM SpSS Statistic, Version 28.0.1.0) was

Fig. 1. Images of the regions of interest used in the thermographic analysis. (A): Scanned area of the ocular globe. Yellow dotted line: boundaries of the scanned eye (TGeye), Blue circles: internal regions of interest within the eye, Green dotted line: reference line from the medial (nasal angle) to lateral (lacrimal angle) canthus. (B): Positions adopted by the pedes and areas where thermographic images were obtained (yellow dotted line). Position of first (DI), second (DII), third (DIII) and fourth (DIV) digits and interdigital spaces. (C): Lateral view of the pes showing the keratinized areas. (D): Lateral view of the pes showing the distal tarsus-metatarsus (dotted pink line).

used for statistical analysis. A descriptive statistical analysis of all thermometric variables was conducted. The study of the correlation between the gold standard technique and the rest of the thermometric measures was based on the Spearman test. The agreement between esophageal temperatures and cloacal, eye or pedes temperatures was investigated using the Bland–Altman analysis with multiple measurements per subject. Also, an agreement study was performed between cloacal thermometry and thermographic measurements. Kolmogorov and Levene tests were conducted to assess whether the differences between the two techniques met the assumptions of normality and homoscedasticity, respectively. As normality ($p < 0.001$) and homoscedasticity ($p < 0.001$) assumptions were violated, data are expressed as median and IQ range and a non-parametric approach was used in the statistical analysis. The median (P50) was used as a measure of bias, while the percentiles P97.5 and P2.5 served as the upper limits of agreement (LoA_u) and lower limits of agreement (LoA_l). These limits were accompanied by their respective 95 % confidence intervals (Upper outer $IC_{95\ \%}$ LoA_u, Lower outer $IC_{95\ \%}$ LoA_l). To compute these values, a bootstrap non-parametric technique based on percentiles was used, adapting the method proposed by Olefson et al. (2015) for repeated-measures design. In this percentile approach, bootstrap resamples were generated for each individual by taking a sample of m_i observations from subject i with replacement. From each of the bootstrap data sets, the LoA and their confidence interval are calculated. These values were calculated based on a random resampling process of the observed data with a simulation of 10,000 samples. Significance was determined at a value of $\alpha = 0.05$.

3. Results

After analyzing the thermographic data, maximum values have been included in the correlation and concordance analysis. The esophageal temperature ranged from 38.4 to 41.70 °C; similar values were obtained from cloacal thermometry (38.0–42.2 °C). In the thermographic study, the maximum temperatures measured in the eyes (35.5–41.3 °C) most closely resembled the esophageal temperatures, than pedes thermographic measurements, which showed more dispersed values of maximum temperatures (20.2–43.3 °C, respectively).

In the thermographic study of the eye, 68.28 % of the Tmax measurements for the whole eye coincided with the Tmax recorded in the medial area of interest, whereas only 0.73 % and 2.2 % corresponded to the central and lateral ROIs, respectively. The remaining Tmax values were located outside the areas delineated by the 7.5-pixel circles. In the pedes, the Tmax values in our study were recorded in feather-free areas where the keratinized skin is exposed. It has been found that Tmax was primarily located in two regions: the base of the claw (51.47 % of cases) and the interdigital space (48.53 %). The primary areas where Tmax was recorded were the interdigital space, either between digits I and II or between digits II and III, depending on the position of the animal's foot, and the base of the claw of digits I, II, or III. The main Tmax area remained constant across all measurements in only 3 of the 8 animals. In the remaining five individuals, variations in the principal Tmax location were observed in some measurements. When Tmax was detected at the base of the claw, the main area (claw base) remained consistent; however, the specific digit involved occasionally alternated.

The results of the association and agreement analysis between the esophageal thermometry and cloacal thermometry, pedes, or eyes thermographic values are shown in Table 1 and Fig. 3. The statistical result using cloacal thermometry as the gold standard was also included in Table 1. The strongest correlation was found between the temperatures measured in the esophagus and the cloaca (Table 1), while a negligible correlation was observed between esophageal thermography and feet thermographic measurements. The agreement study indicated a bias (LoAu-LoAl) of −0.30 (0.90 to −0.76), 0.20 (2.77 to −0.90), 0.30 (3.06 to −0.86), and 0.30 (3.62 to −4.16) °C for Te-Tc, Te-TGeye, Te-TGeyeM, and Te-TGped, respectively. The proportion of Tc, TGped, and TGeye measurements that deviated >0.5 °C or >1 °C of the Te were 15 % (n = 21) or 2.2 % (n = 3), 55.9 % (n = 76) or 36.0 % (n = 49) and 45.6 % (n = 62) or 11.8 % (n = 16), respectively.

4. Discussion

In mammals, esophageal thermometry is considered a reliable gold standard, however, in birds, it is important to consider the following

Table 1
Median, Q1 and Q3 of maximum temperature measured (n = 136), correlation coefficients of each thermographic and cloacal thermometry values with esophageal measures or each thermographic values with cloacal measures, and agreement parameters between esophageal (Te) or cloacal (Tc) measures and thermographically determined values from pedes (TGped), the whole eye (TGeye), and its medial ($TGeye_M$), central ($TGeye_C$), and lateral ($TGeye_L$) aspects in owls (*Bubo bubo*).

Parameter	Te	Tc	TGped	TGeye	$TGeye_M$	$TGeye_C$	$TGeye_L$
Descriptive statistic of the measured temperatures							
Median (°C)	39.2	39.50	39.50	39.20	39.10	37.70	38.30
Q3 (°C)	39.7	40.00	40.10	40.13	40.10	38.70	39.20
Q1 (°C)	38.8	39.10	38.50	38.50	38.30	37.30	37.48
Correlation with esophageal thermometry							
rho		0.761	0.176	0.724	0.736	0.731	0.762
p		<0.001	0.4	<0.001	<0.001	<0.001	<0.001
Agreement with esophageal thermometry							
Bias (°C)		−0.30	0.30	0.20	0.30	1.55	1.10
LoA_u (°C)		0.90	3.62	2.77	3.06	6.42	5.78
LoA_l (°C)		−0.76	−4.16	−0.90	−0.86	0.64	−0.06
Upper outer $IC_{95\ \%}$ LoA_u (°C)		1.54	6.27	3.17	3.23	6.96	6.77
Lower outer $IC_{95\ \%}$ LoA_l (°C)		−1.59	−4.56	−1.00	−0.93	0.50	−0.10
Correlation with cloacal thermometry							
rho			0.435	0.851	0.814	0.599	0.754
p			<0.001	<0.001	<0.001	<0.001	<0.001
Agreement with cloacal thermometry							
Bias (°C)			0.20	0.30	0.50	1.80	1.30
LoAu (°C)			3.96	3.16	3.50	6.77	6.13
LoAl (°C)			−3.66	−0.46	−0.4	0.40	0.30
Upper outer IC95 % LoAu(°C)			6.67	3.57	3.76	7.36	7.17
Lower outer IC95 % LoAl (°C)			−4.06	−0.85	−0.85	−0.16	−0.26

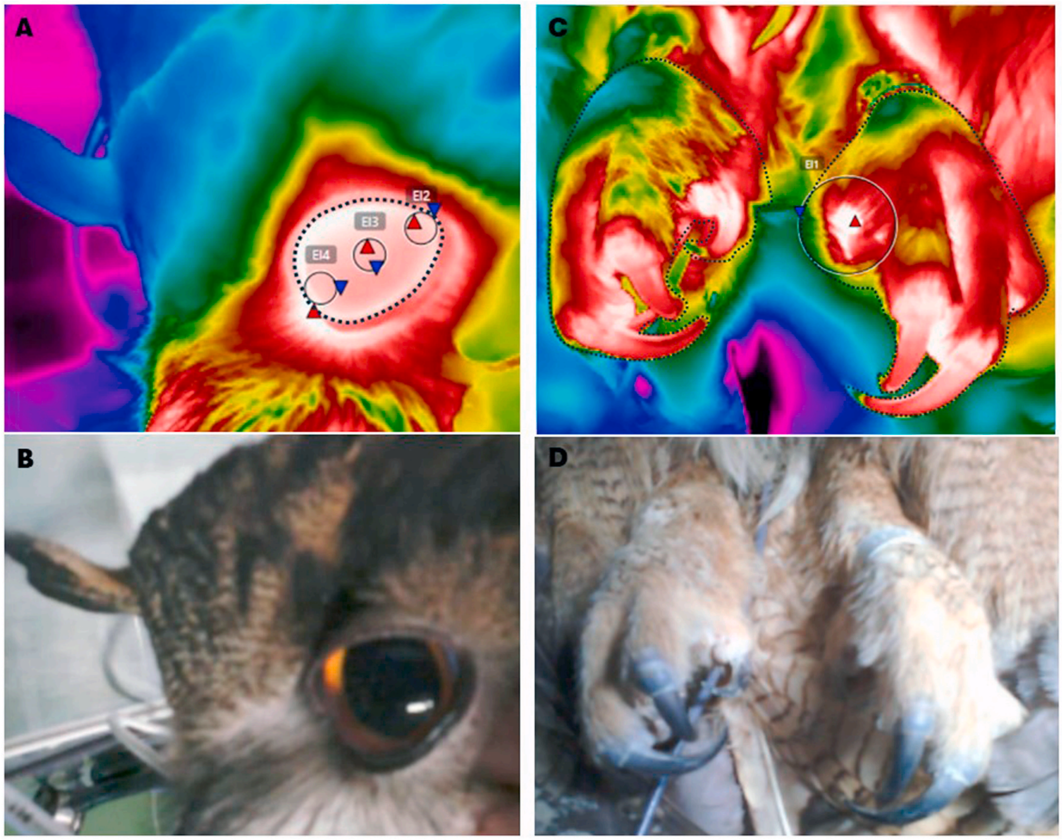

Fig. 2. Infrared thermography and standard images of the eye (A and B) and pedes (C and D) obtained in owls during clinical procedures conducted at the recovery center hospital. Dotted lines indicate the areas of interest scanned in both thermographic images. The circles formed by solid lines indicate additional areas of the images where measurements were taken. The three circled areas were used to assess the thermal values of the lateral ($TGeye_L$), medial ($TGeye_M$), and central ($TGeye_C$) corneal areas of the eyeball. The red dots represent the maximum temperature, while the blue dots indicate the minimum temperature within the marked regions.

anatomical factors. Unlike mammals, the avian esophagus is divided into cervical and thoracic regions. At the thoracic inlet, the esophagus expands to form the crop, which is typically located ventrally in most bird species. While the crop is present in many birds, it is absent in others, such as gulls, penguins, ostriches, and certain raptors and owls like those examined in our study. The crop serves primarily as a food storage organ and may appear spindle-shaped, bilobed, or unilobed. Additionally, a small number of species possess an esophageal sac—a diverticulum or bilaterally symmetrical expansion of the cervical esophagus—which functions as a display structure during the breeding season. The presence of this ingluvies in the esophagus may influence the temperature reading if the probe is not positioned correctly. However, the birds studied lack a crop (Denbow, 2000; König et al., 2009).

Avian body temperature has traditionally been measured at the cloacal mucosa because it is a minimally invasive, cost-effective quick method. However, this technique can be stressful for birds, it is also time-consuming, and poses a risk of cross-contamination. Despite being a consensus to use cloacal or rectal temperatures as proxies for central body temperature due to their accessibility (Gauchet et al., 2022; McCafferty et al., 2015), our results do not fully support that assumption. To the best of our knowledge, there is no information about the appropriate value of the correlation coefficient for the association between pairs of thermometric measurements to be considered a good fit for validating techniques. Following the existing recommendations for the validation of techniques used for other physiological parameters, which apply a cut-off point of rho $\geq$ 0.9 (Brown et al., 2007), we infer that none of the thermometric measurements evaluated in our study reach this level of association. Although cloacal temperature is relatively close to the temperature measured by the esophageal probe and showed a relatively small bias (−0.3 °C), its wide limits of agreement (−0.78–0.90 °C) indicate low accuracy and precision. Different accuracy cut-off points have been described for agreement studies of thermometric techniques. Although the ideal thermometer must be accurate within ±0.1 °C (Hooper and Andrews, 2006), currently, limits of agreement of ±0.5 are considered admissible in veterinary studies (Lamb and McBrearty, 2013; Osinchuk et al., 2014; Sousa et al., 2011). Similarly, in human studies, a mean bias within ±0.2 °C and LoA within ±0.5 °C have been considered clinically acceptable (Cutuli et al., 2021), as normal human circadian temperature variation has been reported to be ±0.5 °C (Ellebrecht et al., 2022). Therefore, in our study, cloacal temperature did not meet the criteria for acceptable clinical accuracy with esophageal temperature. Also, under certain clinical conditions, especially when there is peripheral vasoconstriction resulting from an organic compensatory measure in response to hypovolemia or hypotension (Herrero and Agúndez, 2017), digestive perfusion could be affected, which would alter the cloacal temperature reading. When a circumstance such as the one mentioned above occurs, the correlation would possibly change and, consequently, the agreement.

The degree of association and agreement between esophageal and cloacal temperatures was very similar to that described in griffon vultures (rho = 0.74, [0.0 (−0.5- 2.01) °C]; Hoz de la Iglesia et al., 2024), but lower than the obtained in red kites (r = 0.92, [0.7 (0.1–1.0) °C]; Carballo, 2021. In reptiles, such as awake iguanas and chameleons at any ambient temperature and snakes at high ambient temperature, Cremer et al. (2019) reported that cloacal temperatures are reflective of esophageal temperature. The conclusion obtained in that study was based only on the value obtained for bias (which oscillated between 0.004 and −0.1 °C), but did not take into consideration the limits of

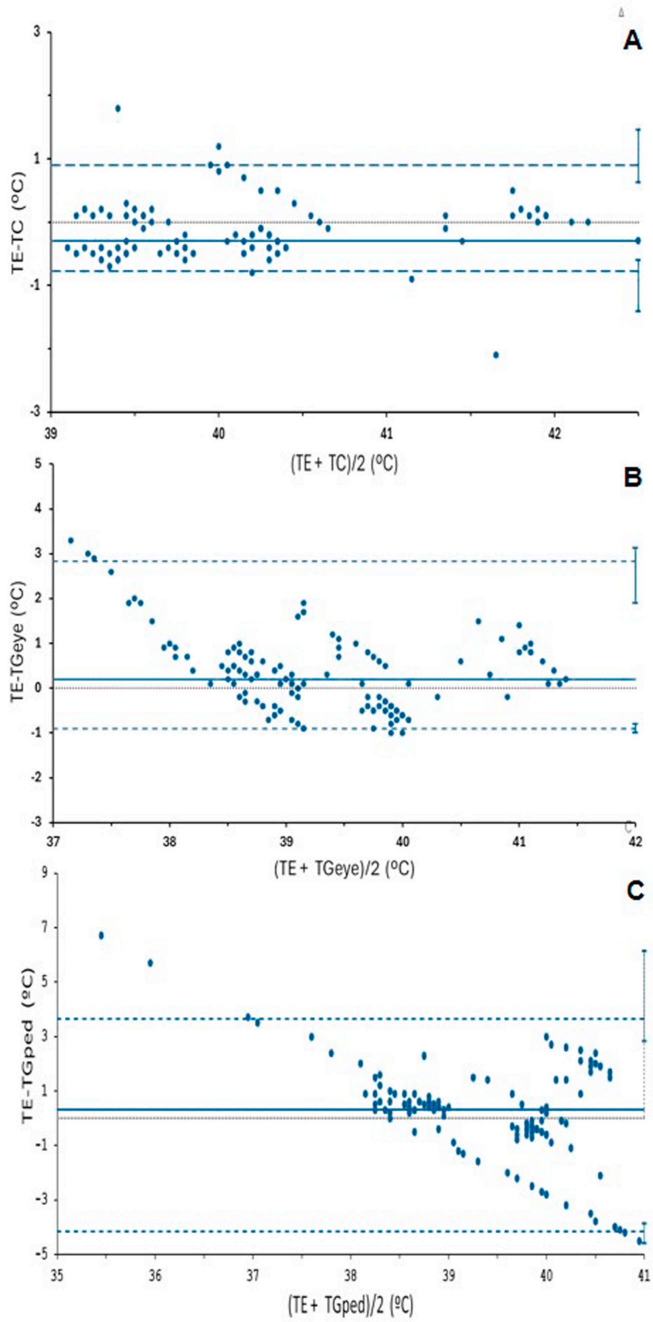

Fig. 3. Bland-Altman plots, obtained from maximum temperature values (n = 136), between esophageal and cloacal (A); esophageal and whole eye (B) or esophageal and pedes (C) temperatures. Zero value (dotted line ·····), bias (solid line) and limits of agreements (dashed lines - -) with their IC 95 % were included. Points shown correspond to individual pairs of observations rather than individual owls. Error bars located in the line of LoA show the 95 % confidence interval of limits of agreement.

agreement. However, McMaster and Downs (2013) found cloacal thermometry to be an inaccurate measure of central body temperature in tortoises.

Cloacal thermometry in birds or reptiles is often considered equivalent to rectal thermometry in mammals. Therefore, if a comparison with mammals is made, a higher concordance than that observed in owls between cloacal and esophageal thermometry was observed in dogs between arterial and rectal thermometry, since 94.3 % of differences are included within ±0.5 °C. The authors indicate that rectal measurements are a good reflection of central body temperature (Greer et al., 2007). However, a recent meta-analysis conducted on human subjects revealed that peripheral thermometers, including rectal thermometry, exhibited clinically unacceptable levels of accuracy compared to pulmonary artery catheter measurements, while esophageal thermometers demonstrated clinically acceptable accuracy. The study noted that rectal thermometry had high variability and wide limits of agreement, making it an unreliable method for accuracy temperature measurement (Cutuli et al., 2021).

Liles and Di Girolamo (2023) reported that temperature obtained via cloacal mucosal surface in birds is likely to underestimate central body temperature. However, considering the technique's biases, our results suggest that cloacal thermometry might be a valuable tool in wild birds clinically.

Infrared thermography is a tool of great interest in wild species since it is a non-invasive and passive method, but it is important to know to what extent it provides information on central body temperature. In the present study, the best agreement with central body temperature was associated with eye thermography. Different studies have used infrared thermography to measure eye temperature in different avian and mammalian species, finding that it may reflect changes in body temperature in response to events and stimuli (Church et al., 2014; Jerem et al., 2015; Kim and Cho, 2021; Arfuso et al., 2022). Several thermographic studies have shown that ocular thermography is better correlated with central body temperature compared with other anatomical regions [Kim and Cho, 2021]. This association is attributed to the proximity of the orbit to the brain, as well as the rich blood supply it receives (Church et al., 2014). To our knowledge, there is only one study conducted in kites (*Milvus milvus*) that is focused on analyzing the degree of agreement of TGeye with central body temperature (Carballo et al., 2022). Compared with the results obtained for owls, kites showed a lower correlation between Te and TGeye (r = 0.68) than those observed in the present study. Also, there is a higher agreement between Te and TGeye observed in owls than that reported in kites [2.4 (1.1–4.61) °C]). These differences between kites and owls may be related to differential ocular anatomo-physiological structures. Perhaps the large size and high exposure of the eyeball of the owl could have influenced these results. However, the pecten, which is the most irrigated area of the bird's eye, is most developed in diurnal raptors (Beckwith-Cohen et al., 2015). Additionally, stressful situations may alter the correlation and agreement between ocular thermography and esophageal temperature, by reducing the temperature of the eye as it has been shown in wild blue tits (Cyanistes caeruleus) (Jerem et al., 2015).

Several studies use thermography as a method to determine which area of the bird's surface best provides information about its temperature. Facial infrared thermograms of red-footed boobies (*Sula sula*) showed that the highest facial temperatures, located at the bill's corner and the internal canthus of the eye, provided the best relationships with central body temperatures (Gauchet et al., 2022). In domestic pigeons (*Columba livia domestica*), stress-induced changes in surface temperature are most noticeable at the bill rather than in the eye. Thermal responses at the bill provide sufficient resolution to detect and quantify differences in responsiveness. Therefore, bill temperature is a more reliable indicator of stress-induced thermal responses than the eye region in pigeons (Tabh et al., 2021). The bill area was not included in our study for several reasons. In the present study, a previous scan of the facial area revealed that the highest temperature was located in the eyes. While the bill has been used in some bird species for thermographic measurements due to its uninsulated nature and role in heat dissipation (Janse van Vuuren et al., 2020), we determined that it was not a useful area for thermography measurements in eagle owls during our study, conducted in anesthetized birds at high room temperature. This may be because eagle owls have short bills with a small surface area, as has been seen in other raptor species (Rogalla et al., 2021) and unlike other avian species in which the long beak plays a relevant role in heat exchange (Van de Ven et al., 2016; Janse van Vuuren et al., 2020). Additionally, the proximal area of the eagle owl's bill is partially covered by feathers, isolating the zone and thus diminishing the accuracy of thermography measurement in that area.

The large discrepancies between feet thermographic measurements compared with esophageal temperatures preclude the use of this area in clinical practice. The reason for these poor associations and agreement may be related to feather-impeded infrared emission detection at some sites and the epidermic characteristics of the feet of owls. Eurasian eagle owls present strong feet with powerful claws, with which they kill their prey by stabbing and crushing. The toes of these owls are feathered as far as the distal interphalangeal joints (Korbel and Liebich, 2009). The Tmax was consistently recorded in a region where the natural arrangement or separation of feathers allowed access to the skin surface (interdigital space or base of the claw). It is noteworthy that the areas of the interdigital space are irrigated by prominent arteries (medial at the digit I, lateral at digit II) and prominent veins (medial at digits II and III) (Schwehn et al., 2024). In contrast with our results, a higher correlation and agreement between esophageal and feet thermography was found in kites ($r > 0.90$; [−0,20 (0,98 to −0,80) °C], respectively) (Carballo et al., 2022). This discrepancy could be due to the different feet interest area used for thermography (plantar zone in kites; dorsal zone in owls) or the presence of feathers and a greater stratum corneum on the pelvic limbs of owls. The importance of the feathers in the response obtained is consistent with the fact that, in owls, the area of the pedes where the maximum temperature is recorded is the interdigital space between fingers 1 and 2, which, as can be seen in Fig. 1, is an area where the feathers are reduced. Furthermore, in images taken of the bird's body, the area covered with feathers always showed lower temperature values than the bird's esophageal temperature. In addition, the environmental temperature in the study conducted in kites was set and controlled at 29 °C instead of 27 °C. It is logical to expect that ocular thermography would show greater concordance with esophageal temperature than foot thermography, given that the latter is highly dependent on ambient temperature (McQueen et al., 2023). It should also be noted that, in clinical situations that modify arterial blood pressure, blood flow to the eye could be altered, which may affect the temperature measured by thermography. This has been observed in pigeons, where it was found that, when mean arterial blood pressure (MAP) ranges between 50 and 80 mmHg, choroidal blood perfusion is maintained, but compensation fails with MAP values under 45 mmHg (Reiner et al., 2003).

Certain thermographic studies carried out in mammalian species used rectal mucosal surface thermometry as the gold standard. In these studies, the most common body areas evaluated by thermography are the eye (pig: Barbiere et al., 2021; horses: Johson et al., 2011, Kim and Cho, 2021, Ruston et al., 2015; sheep: Arfuso et al., 2022, cat: Giannetto et al., 2021 and dog: Zanghi, 2016) and the ear (dog: Zanghi, 2016; cat: Nutt et al., 2016).

In our study, a stronger correlation between cloacal thermometry and TGeye was observed compared to the correlation found between rectal thermometry and eye thermography in sheep under non-stressful conditions (Arfuso et al., 2022). A weak association between rectal and eye temperature was also found in cats ($r = 0.3$; Giannetto et al., 2021), rabbits ($r = 0.39$; Jaén-Téllez et al., 2021), dogs ($r = 0.38$ or $r = 0.67$, sedentary or with exercise, respectively; Zanghi, 2016) and horses ($r = 0.30$; 0.34 and 0.21 for lacrimal sac, medial and lateral canthus respectively; Kim and Cho, 2021).

It is important to note that the biases and limits of agreement obtained in sheep are higher than those observed in our study and exceed the cut-off points for optimal agreement (±0.5 °C). Additionally, ocular thermography in sheep loses accuracy when the animal is stressed due to handling and shearing. This loss of accuracy was attributed to acute stress causing peripheral vasoconstriction (Arfuso et al., 2022). This finding could be explained by the effect of stress on eye temperature, as demonstrated by Jerem et al. (2015). Ear temperature was found to be a more accurate reflection of rectal temperature than eye temperature in dogs. The agreement obtained between rectal and ear temperatures [0.1 (0.5 to −0.35) °C] is among the results that closely align with the satisfactory range found in the literature. In contrast, the agreement between rectal and eye temperatures was poorer [1.0 (0.05–1.9) °C] and demonstrated no accuracy (Zanghi, 2016). However, the influence of devices and the method of temperature measurement should be taken into account, since ear temperature was detected by infrared detection (Pet-Temp PT-300) of the tympanic membrane and ear canal and only the eye temperature was obtained with a portable thermal camera.

The differences among these correlation and agreement studies may be attributed to varying environmental conditions, devices or measurement areas in each investigation, as well as potential biases identified in the case of Giannetto et al. (2021), who, to our knowledge, are the only ones to have conducted a prior ophthalmologic assessment excluding individuals with ocular abnormalities. Additionally, the measurement area varies between studies among the medial canthus of the eye (rabbits, Jaén-Téllez et al., 2021), the lacrimal sac, the medial and the lateral canthus (Kim and Cho, 2021), the entire eyeball without distinguishing between ocular zones (dogs, Zanghi, 2016; cats, Giannetto et al., 2021), or the entire and partial areas of the eyes, similarly to the approach used in our study (sheep, Arfuso et al., 2022). The better agreement between eye thermography and rectal thermometry at the medial canthus of the eye has been attributed to the presence of the lacrimal caruncle (Kim and Cho, 2021). It has also been suggested that thermographically measured temperatures of the lacrimal caruncle region of the eye may correlate with central body temperature, in essence serving as a proxy for central body temperature in cattle (Church et al., 2014); however, Costa et al. (2023) did not recommend to use the inner eye canthus temperature as a substitute for rectal temperature for measuring central body temperature in human beings. Studies conducted in sheep and horses (Kim and Cho, 2021; Arfuso et al., 2022) assessed which area of the eye most closely resembles the gold standard thermometry method similar to what was performed in the present research. In horses, the medial canthus temperature was the closest to the rectal temperature, and, therefore, it was suggested as the recommended place for thermographic temperature measurement (Kim and Cho, 2021). In sheep, under non-stressful conditions, when rectal thermometry is used as the gold standard, the best agreement was observed with the entire eye [0.66 (−1.20-1.40) °C], rather than the medial canthus [1.58 (0.16–3.00) °C] (Arfuso et al., 2022); however, Bakker et al. (2024) reported that, the whole eye exhibited the lowest level of agreement, indicating that it is a less reliable site for infrared thermography estimation compared to the lacrimal caruncle or the medial canthus. In owls, the best agreement between esophageal thermometry and thermography measures was found with the entire eye and the medial portion of the eye. It is worth noting that the high temperatures recorded in the medial canthus of the eye are not related to the lacrimal gland, which is absent in owls, but rather to the gland of the third eyelid, located near the base of the nictitating membrane at the nasal angle of the eye (Reese and Liebich, 2009). The differences in design between the studies could be a limiting factor when making comparisons. When comparing the thermographic results obtained from the eye and the pedes, the medial region of the eye appears to be the most reliable site for a rapid thermographic measurement (directly from the camera), as it demonstrates a closer agreement with the esophageal temperature than pedes thermography and corresponds to the area exhibiting the highest proportion of Tmax readings within the ocular surface.

Other limitations of our study, which should be taken into account when extrapolating our results, are that these temperature values were obtained in anesthetized birds at a high ambient temperature (27 °C), considered optimal for an operating room intended for avian species; however, methods could show lower concordance when the ambient temperature is reduced or in other clinical circumstances. The level of accuracy of the thermographic camera is low, with ±2 °C or 2 % of the recorded measure. This value is higher than the accuracy of the esophageal or cloacal probes and involves a potential risk of delayed detection of hypo- or hyperthermia, which could have clinical implications.

Another limitation of the study is the variability in the pedes position and the location where Tmax were registered. The variations in the Tmax location among measurements are likely attributable to subtle changes in foot or feather position. Since the measurements were obtained during routine clinical procedures at the rehabilitation center, it was not possible to maintain an identical foot position throughout the sampling period. Among the factors that could influence this, we highlight the dependence of body thermography on ambient temperature, particularly critical in avian appendages such as the beak, legs, or crest. In addition, the resolution of the camera used, the distance at which the photograph is taken, and the loss of accuracy outside an optimal temperature range must also be considered (Playà-Montmany and Tattersall, 2021; McQueen et al., 2023). Further constraints of our research could be related to an increase in basal temperature due to stress that might have been produced by handling prior to anesthetic induction. However, all animals showed a basal temperature within the physiological range of the species.

5. Conclusion

From a thermometric standpoint, a difference of >0.5 °C with the thermometric reference technique is considered unacceptable. As a result, neither cloacal nor thermographic temperature measurements are interchangeable with esophageal temperature values. Therefore, their use is not recommended as reliable methods for accurate body temperature measurement. However, this may not pose a problem if the differences between central body and cloacal temperatures follow a consistent pattern and reference intervals are established for each measurement site (Liles and Di Girolamo, 2023).

Among the thermographic regions studied, the eye provide a closer agreement with esophageal temperature than with the temperature measured at the pedes. However, owing to the inherent limitations of the technique, including its dependence on ambient temperature and, in particular, its limited accuracy, its clinical applicability may be constrained. Furthermore, it seems to be applicable only under controlled circunstances, where the anesthetized bird is in an operating room with a high ambient temperature. Moreover, the results of this study help us understand how far we are from established cutoff points, highlighting the need for further research on central body temperature measurement, especially in species that are particularly sensitive to handling. It is also worth mentioning the need to evaluate the speed of response of each of the thermometric methods to rapid temperature changes.

CRediT authorship contribution statement

Abril Fernández: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Casilda Rodríguez:** Writing – review & editing, Project administration, Methodology, Formal analysis, Conceptualization. **Virginia Moraleda:** Resources, Methodology, Investigation, Formal analysis. **Irene López:** Resources. **Laura Suárez:** Investigation. **Natalia Pastor:** Investigation. **Samanta Waxman:** Writing – review & editing, Conceptualization. **Fernando González:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The raw data required to reproduce the above findings are available to download from https://hdl.handle.net/20.500.14352/122370.

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