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Research article

Infrared thermography as a diagnostic tool for pododermatitis in captive greater flamingos (*Phoenicopterus roseus*)

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Abstract

This cross-sectional study investigated the use of infrared thermography as a diagnostic tool for pododermatitis in captive greater flamingos (*Phoenicopterus roseus*). Photographs and thermal images were obtained for 775 feet from 408 flamingos held at three UK zoological collections. The feet were divided into eight regions, which were assigned a score for hyperkeratosis, fissures, nodules and papillomatous growths according to a previously defined scoring system. Minimum, mean and maximum temperatures were recorded for each region. 97 feet (12.5%) were scored as normal (no lesions or only mild hyperkeratosis), whilst 678 (87.5%) were scored as abnormal. It was found that 99.9% (95% confidence interval (CI): 99.3–100%) of the scored feet exhibited hyperkeratosis, 61.7% (95% CI: 58.2–65.1%) fissures, 16.0% (95% CI: 13.5–18.8%) nodules and 38.5% (95% CI: 35.0–42.0%) papillomatous growths. Thermal data assessed using general linear mixed effect modelling showed that regional and individual bird temperature differences accounted for most of the temperature variation, but there was a statistically significant ($P<0.05$) difference between regions with nodules versus regions without when using maximum temperatures. Intra- and inter-foot variation, using a regional correction factor and ankle temperatures, was assessed for 272 birds, where temperature distributions for each lesion type were compared with that of normal regions using t-tests. A statistically significant difference ($P<0.05$) was found between corrected values for regions with hyperkeratosis and papillomatous growths compared with normal, but no difference was found for fissures or nodules. Despite the differences found, the results suggest that infrared thermography may not be a practical diagnostic tool for pododermatitis in flamingos due to wide temperature variations between and within normal feet and a great degree of overlap of temperatures between normal and abnormal feet.

Introduction

Pododermatitis, foot pad dermatitis or bumblefoot, is a common skin disease in a wide range of bird species characterised by abrasion, ulceration, vascular damage, inflammation and bacterial colonisation of the skin on the plantar surfaces of the feet (Remple and Al-Ashbal 1993). Clinical signs can be mild and only apparent as skin lesions on the weight bearing surfaces of the feet, but in severe cases the disease can be debilitating, causing chronic lameness or lead to septicaemia and death. Whilst the condition does occur in wild birds, it is mostly a disease of captive animals and can have significant welfare and economic implications in captive populations (Remple and Al-Ashbal 1993; Wyss et al. 2013). Captive flamingos (*Phoenicopterus spp.*), commonly kept in zoological collections around the world, are frequently affected by pododermatitis. A previous study by Nielsen et al. (2010) demonstrated that almost 100% of all examined captive greater flamingos across several European and American zoos showed some degree of foot changes. Currently, initial diagnosis of pododermatitis is based on visual inspection of the feet for the presence of classical lesions. The limitation of this is that the condition will thus only be detected once the lesions have formed and the disease is well established. The ability to detect subclinical disease, or to distinguish between active and inactive (healed/fibrosed) lesions would be highly beneficial, both for deciding between treatment options and for implementing relevant husbandry changes at a timely stage.

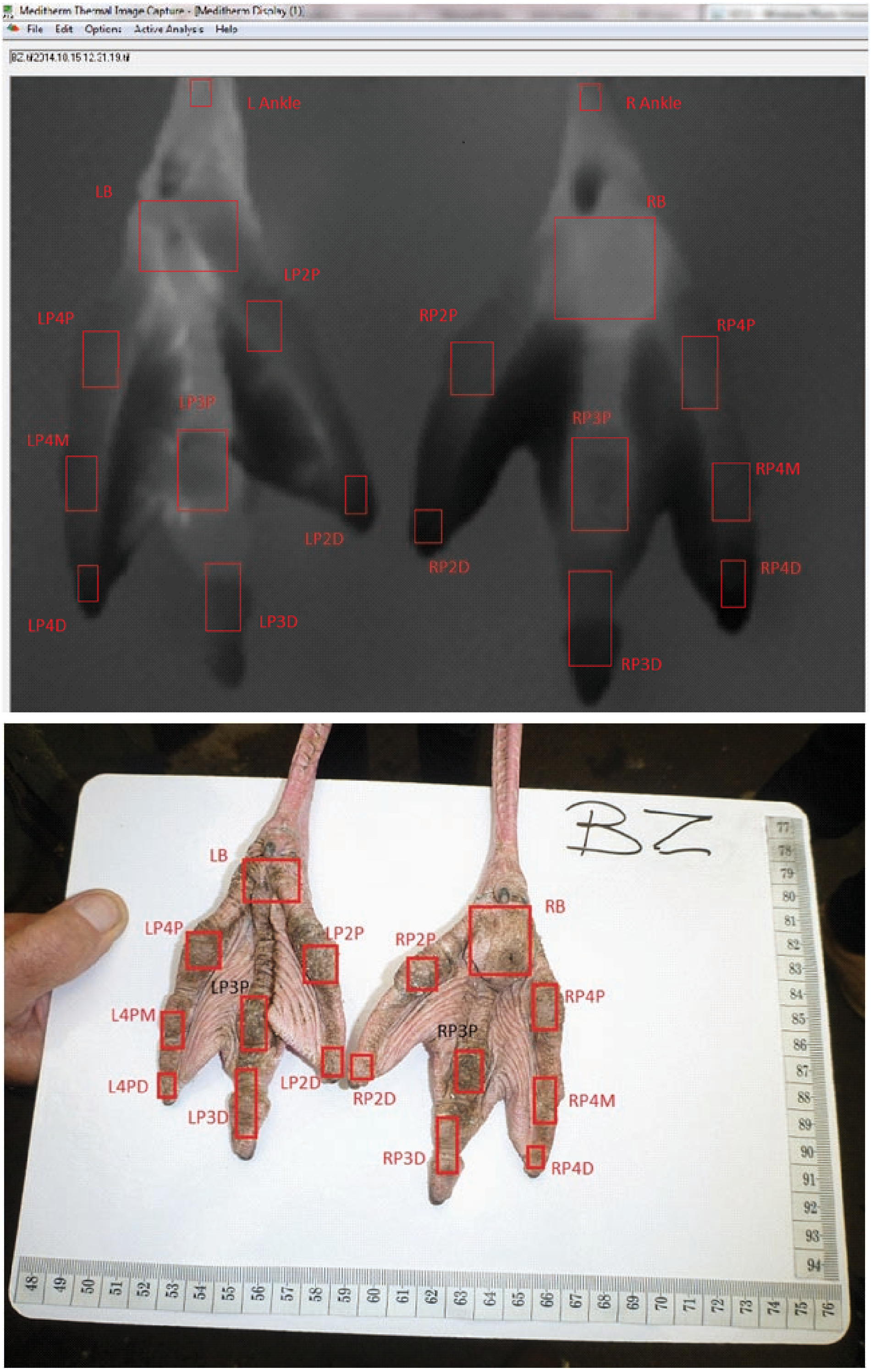

Figure 1. Thermal image and normal photograph showing the 8 regions of interest (ROI) of a flamingo foot. L and R denotes left and right foot respectively. B – base (metatarsal pad, excluding the hallux); P2P – proximal phalanx II (1st interphalangeal joint); P2D – distal phalanx II (2nd interphalangeal joint); P3P – proximal phalanx III (1st interphalangeal joint); P3D – distal phalanx III (2nd/3rd interphalangeal joint); P4P – proximal phalanx IV (1st interphalangeal joint); P4M – middle phalanx IV (3rd interphalangeal joint); P4D – distal phalanx IV (4th interphalangeal joint).

Infrared thermography (IRT), or infrared thermal imaging, is a safe, non-radiating and non-invasive imaging tool that can be used to remotely detect surface temperatures on the body. It has gained popularity as a diagnostic tool with a wide range of medical and veterinary applications and is frequently used for the detection of localised or generalised surface temperature changes caused by inflammation, disease, injury and certain physiological processes (Cilulko et al. 2013). Previous studies have suggested that IRT can also be a useful tool to detect subclinical pododermatitis in poultry and that it may be a more sensitive technique than visual inspection alone, however this did not seem to be the case in penguins (Wilcox et al. 2009; Duncan et al. 2016).

The aim of this cross-sectional study was to establish whether a relationship exists between visible foot lesions in captive greater

Table 1. Description of lesion scores used for visual foot scoring (Nielsen et al. 2010).

Lesion type		Definition
Hyperkeratosis	1	Flattened or mildly enlarged skin pattern
	2	Widening of joint, disruption or marked enlargement of skin pattern, markedly thickened skin
Fissure	1	Superficial crack, less than 2 mm deep
	2	Deep crack, more than 2 mm deep
Nodule	1	Obvious swelling which appears three dimensional in nature
	2	Swelling with a necrotic centre
Papillomatous growth	1	Single, finger-like proliferations
	2	Clusters of proliferations, cauliflower-like

flamingos (*Phoenicopterus roseus*) and thermal image patterns of the plantar surface of the foot in order to investigate the potential utility of IRT as a diagnostic tool for pododermatitis in flamingos.

Methods

Study population

The study population consisted of 408 captive greater flamingos from three zoological collections in England that were scheduled to have a routine annual health check under manual restraint during the study period. The entire flock (51, 268 and 89 birds respectively) from each collection was included. Of these, 121 were male, 129 were female and 158 were of undetermined gender. The birds ranged from 3 months to 59 years (median age 9 years) and weighed between 1.2 and 4.64 kg (mean 2.74 ± 0.56 kg).

Data collection

Photographic and thermographic data collection was carried out opportunistically during scheduled annual catches in October/November 2014 and March 2015 in order to avoid unnecessary additional handling stress. Two handheld Meditherm Med2000 IRIS 7.5 cameras were used to collect thermal images of the plantar surface of the flamingos' feet, whilst photographs were taken using a handheld Panasonic Lumix DMC-FT3 camera. During one catch (collection 1), the thermal cameras were mounted on a stand due to the long duration of the catch. All thermal images were taken indoors, away from direct sunlight or draughts. The ambient temperature in the sheds where thermography was carried out on the catch days was between 9.2 and 14.9°C and the humidity 64–80%, measured using a separate digital thermometer/hygrometer.

The flamingos were held on straw or wood crumble bedding (Aquamax, UK) without access to water for a minimum of 30 minutes prior to imaging to allow the feet to dry. Each bird was individually restrained and presented for imaging by a keeper. Care was taken to not touch the flamingos' feet prior to thermal imaging, and in cases where the soles of the feet had been touched, the bird was excluded from the study. Any gross contamination, like bedding, was carefully removed with a dry swab when necessary. To provide an even background, thermal images were taken with the feet held against a handheld, non-reflective A3 size plywood or cork board at a distance between 35 and 75 cm from the camera. It was not possible to control how long the birds had been restrained off the ground prior to imaging.

Data analysis

Thermal image analysis

Thermal image analysis was carried out using WinTES2 software (version 1.00.0030, Compix Inc., USA 2009). A temperature range of 16°C was set for each image and the greyscale colour map was used to allow optimal visualisation of the feet. The temperature range was then adjusted to best fit the absolute temperatures of the individual foot, so the feet were as visible and clear as possible. Due to software limitations, a rectangle method was used for temperature extraction. Each foot was divided into eight operator-determined regions of interest (ROI) as shown in Figure 1. These ROI were chosen to capture regions of the metatarsal pad (the heel/base) and the digits most commonly showing or having foot lesions during examination of the first 140 birds. Minimum, mean and maximum temperature readings were recorded using the "Data" function in WinTES2, where a rectangle was placed over each ROI. The rectangle was placed over as much of the digit as possible, while excluding the background and interdigital webbing when possible. Images of insufficient quality or ROI that were obscured or indistinguishable from surrounding structures were recorded as missing observations. Additionally, temperatures were recorded from the plantar surface of the distal tarsometatarsus just above the hallux (from here on termed "ankle") in 272 randomly selected birds, to assess inter-foot temperature variability.

A rectangle approximately two thirds of the width of the leg was placed on a level just above the hallux and minimum, mean and maximum temperatures recorded. The minimum, mean and maximum regional thermal data was analysed in its raw form as absolute temperatures, as a ratio of ROI to ankle temperatures and as the difference between ankle and ROI (ΔT).

Visual scoring

The flamingo feet were visually scored from regular photographs using a modified version of the scoring system developed by Nielsen et al. (2010). All images were scored blindly in relation to the thermal image scores. Only lesions within the ROI used for thermal image analysis were scored to allow direct comparison between the two scoring systems. Each area was given a severity score of 0 (no lesion), 1 (mild) or 2 (severe) and a lesion type score of hyperkeratosis (H), fissures (F), nodules (N) and papillomatous growths (P) according to the criteria described by Nielsen et al. (2010) and as outlined in Table 1. Each area could be affected by more than one lesion type. ROI obscured by excessive contamination or other toes were recorded as missing observations. Lesions classed as mild hyperkeratosis (H1) were ignored and counted as normal due to their high prevalence (99.6% of feet) and assumed inconsequential nature (Nielsen et al. 2010). ROI were grouped according to the lesion types present and basic prevalence statistics obtained.

Statistical methods

Thermal data were analysed in two ways: by using general linear mixed effect models and by comparing temperature distributions across all ROI with and without lesions, using values corrected for inter- and intra-foot variability. Mean foot temperature

Table 2. Regional correction factors for minimum, mean and maximum temperatures based on normal feet.

	Average of Min_Region/ Min_Foot	Average of Mean_Region/ Mean_Foot	Average of Max_Region/ Max_Foot
Base	1.09	1.12	1.19
D2P	0.99	0.98	0.97
D2D	0.94	0.93	0.91
D3P	1.01	1.01	1.03
D3D	1.02	1.05	1.05
D4P	0.99	0.98	0.97
D4M	0.98	0.96	0.95
D4D	0.96	0.95	0.92

distributions were also assessed between catch days and between feet scored as normal versus abnormal. All statistical tests were carried out using Minitab 17 Statistical Software (Minitab, Inc., USA, 2014), with a statistical significance level placed at $p < 0.05$.

Assessing temperature variance between sampling days and between normal and abnormal feet

To assess the differences in recorded temperatures between sampling days, the variance of mean foot temperatures between the flocks was tested using one-way Welch ANOVA. Due to outliers and non-normal sample distribution using Anderson-Darling test for normality (collection 1, n = 504, AD = 8.439, $p < 0.005$; collection 2, n = 174, AD = 2.004, $p < 0.005$; collection 3, n = 97, AD = 3.272, $p < 0.005$), values were log10 transformed and ANOVA was run on transformed as well as original data. Both tests yielded the same results, so only results for the original data are reported. Difference in mean foot temperature distributions between normal and abnormal feet were also assessed, using the two-sample t-test.

General linear mixed effect modelling

Thermal data was analysed using a general linear mixed effect model, taking into account bird ID, foot regions and the presence/absence of each of the four lesion types. Tests were run for minimum, mean and maximum absolute temperatures, region to ankle ratio values and for ΔT values, comparing the presence versus absence of each of the four lesion types.

Analysis of temperature distributions corrected for inter- and intra-foot variability

To overcome the effect of normal heat dispersion between the base of the foot and the digits (Wilcox, Patterson and Cheng 2009), minimum, mean and maximum regional correction factors were created based on the ratio between the mean temperatures of each region and the mean foot temperature using all normal feet (Table 2). The correction factors were applied to all raw data for which ankle temperatures were available, along with an ankle correction using the formula:

$$\frac{T_{region}}{Correction\ factor} - T_{ankle}$$

The corrected temperature values for hyperkeratosis, fissures, nodules and papillomatous growths were assessed against corrected values for normal regions using the two-sample t-test. Only regions with a single lesion type present were assessed using this method to avoid confounding effects of multiple lesion types.

Results

Descriptive statistics

A total of 141 ROI and 41 whole feet were recorded as missing observations. Scores and temperatures were obtained for 6,059 ROI from 775 feet, following all exclusions. Of these, 97 (12.5%) feet were scored as normal (no lesions other than H1 or healed fissures) and 678 (87.5%) as abnormal (at least one ROI affected by any lesion other than H1 or healed fissures). Across the three flocks, 99.9% of the scored feet were affected by hyperkeratosis of any severity, 61.7% by fissures, 16.0% by nodules and 38.5% by papillomatous growths. For the severe scores, 50.7% of feet had hyperkeratosis, 33.9% had fissures, 6.2% had necrotic nodules and 9.7% had papillomatous growths (Table 3). There was a marked difference in the regional distribution of each lesion type. Hyperkeratosis, nodules and papillomatous growths tended to occur more frequently on the proximal weight-bearing surfaces of the foot, whereas fissures were relatively more common distally. The regional lesion prevalence (with 95% CI) is summarised in Table 4.

Temperature distributions

Across all three flocks, recorded regional temperatures varied from 2.67 to 32.93°C, with a statistically significant difference in the mean foot temperature between the three flocks (Figure 2, $F_{2,252}$=153.6, $p < 0.001$). Mean temperatures were 9.29°C±3.92°C for collection 1 (n=504); 14.87°C±3.70°C for collection 2 (n=504); and 12.49°C±2.96°C for collection 3 (n=97), with feet generally being warmer on the warmer catch days. There was no statistically

Table 3. Percentage (and 95% CI) of feet with each lesion type in each flock and in total.

	n	H1+H2 (%)	H2 (%)	F1+F2 (%)	F2 (%)	N1+N2 (%)	N2 (%)	P1+P2 (%)	P2 (%)
Collection 1	504	99.4 (98.9–100.0)	45.8 (41.4–50.3)	57.5 (53.1–61.9)	31.0 (26.9–35.2)	14.1 (11.2–17.4)	7.7 (5.6–10.4)	38.5 (34.2–42.9)	10.7 (8.2–13.7)
Collection 2	174	100.0 (>98.3)	57.5 (49.8–64.9)	85.1 (78.9–90.0)	54.6 (46.9–62.1)	20.1 (14.4–26.8)	4.0 (1.6–8.1)	41.4 (34.0–49.1)	8.0 (4.5–13.1)
Collection 3	102	100.0 (>97.0)	63.9 (53.5–73.4)	41.2 (31.3–51.7)	12.4 (6.6–20.6)	18.6 (11.4–27.8)	2.1 (0.2–7.3)	33.0 (23.8–43.3)	7.2 (3.0–14.3)
Total	775	99.9 (99.3–100.0)	50.7 (47.1–54.3)	61.7 (58.2–65.1)	33.9 (30.6–37.4)	16.0 (13.5–18.8)	6.2 (4.6–8.1)	38.5 (35.0–42.0)	9.7 (7.7–12.0)

Table 4. Prevalence (and 95% CI) of each lesion type (any severity) per region, all flocks.

	n	H (%)	F (%)	N (%)	P (%)
Base	771	77.2 (77.0–80.1)	6.7 (5.1–8.8)	4.2 (3.0–6.0)	8.6 (6.7–10.8)
Proximal D2	773	98.6 (97.5–99.3)	28.2 (25.1–31.5)	3.8 (2.5–5.3)	27.3 (24.2–30.6)
Proximal D3	661	95.5 (93.6–96.9)	18.9 (16.0–22.1)	5.5 (3.8–7.5)	19.1 (16.1–22.3)
Proximal D4	774	96.5 (95.0–97.7)	16.7 (14.1–19.5)	3.6 (2.4–5.2)	15.5 (13.0–18.2)
Middle D4	775	51.4 (47.8–54.9)	21.7 (18.8–24.7)	0.0 (<0.4)	2.2 (1.3–3.5)
Distal D2	771	49.0 (45.4–52.6)	6.2 (4.6–8.2)	0.0 (<0.4)	0.9 (0.4–1.9)
Distal D3	763	76.2 (73.0–79.1)	25.0 (22.0–28.3)	1.1 (0.5–2.1)	10.4 (8.3–12.7)
Distal D4	771	39.4 (36.0–42.3)	16.7 (14.2–19.6)	0.0 (<0.4)	1.0 (0.4–2.0)

significant difference in the mean foot temperature of feet scored as normal compared to abnormal (t_{128}=0.79, p=0.431). The mean foot temperature of normal feet ranged from 4.19°C to 23.90°C (mean 10.63°C ± 4.23°C), whereas abnormal feet ranged from 3.14°C to 26.42°C (mean 10.99°C ± 4.47°C).

The mean and SD of the minimum, mean and maximum raw temperatures (pooled from all regions and uncorrected for inter-foot variation) were calculated for all possible lesion combinations. These values, along with the number of regions affected by each lesion combination, are summarised in Table 5.

General linear mixed effect models

Taking into account bird ID, foot region and presence/absence of each lesion type, there was no statistically significant evidence for a lesion type effect on temperature variance, except from when looking at maximum absolute temperatures ($F_{1,5662}$=4.43, p=0.035) and maximum region to ankle ratio values ($F_{1,3717}$=3.92, p=0.048) for nodules. Most of the variance was instead associated with foot region and bird ID (p<0.001 for all values) rather than with the presence of each lesion type.

Corrected temperature distributions

There were no statistically significant differences between regions with fissures or nodules when compared against normal regions, using values corrected for region and ankle. However, hyperkeratotic regions were statistically significantly colder than normal regions for minimum (t_{223}=-3.21, p=0.002), mean (t_{233}=-3.49, p=0.001) and maximum (t_{221}=-2.21, p=0.028) values. Papillomatous growths were also significantly colder than normal regions when assessing minimum (t_{143}=-2.16, p=0.032) and mean (t_{144}=-2.33, p=0.021), but not maximum values. The distributions of the corrected values for each lesion type are displayed in Figure 3.

Discussion

The working hypothesis for this study was that hyperkeratosis would appear colder than normal tissue due to epidermal thickening and ischaemia, resulting in reduced blood flow and heat emission to the skin surface. Conversely, fissures, nodules and papillomatous growths were hypothesised to appear warmer due to associated active inflammation. Although the high prevalence of foot lesions (>99%, consistent with previous studies by Nielsen et al. 2010) made it difficult to assess lesions against completely normal feet, the results from this study demonstrate that a statistically significant difference exists between normal regions and regions with hyperkeratosis and papillomatous growths using t-tests for the corrected values, as well as between areas with and without nodules using general linear mixed effect models. However, the results also show that there was a more

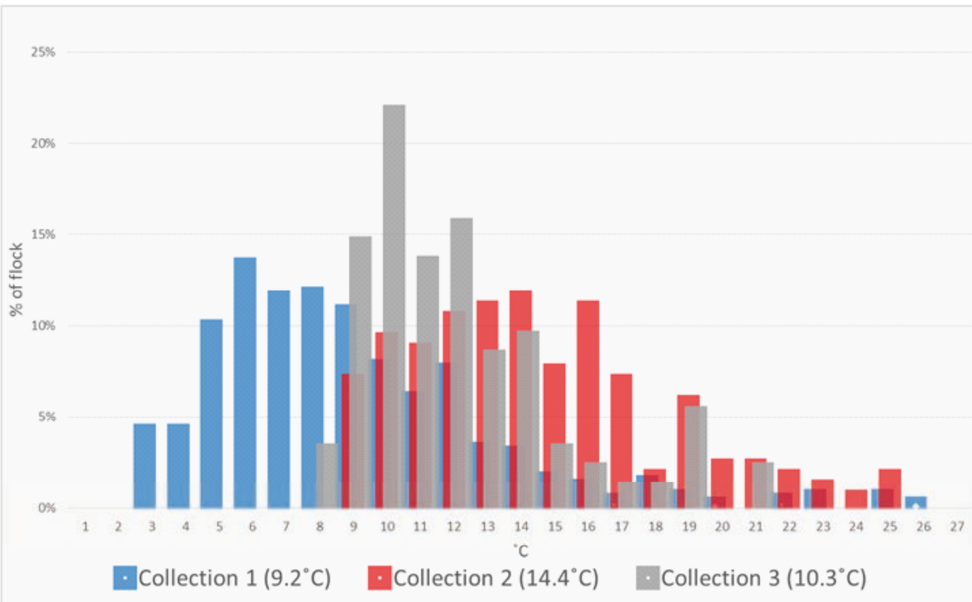

Figure 2. Mean foot temperature distribution for each of the three flocks. The minimum ambient temperature recorded on each catch day is displayed in brackets.

Table 5. Number of regions affected by each lesion combination (any severity except where stated), including regions scored as normal, with the mean temperatures ± SD for minimum, mean and maximum values.

	n	Min temp (° C)	Mean temp (° C)	Max temp (° C)
Normal	4277	10.13 ± 4.19	10.87 ± 4.62	11.86 ± 5.17
H (score 2)	294	10.94 ± 4.14	11.66 ± 4.42	12.90 ± 5.02
F	641	10.51 ± 4.39	11.23 ± 4.79	12.16 ± 5.27
N	27	9.89 ± 3.58	10.73 ± 3.87	12.13 ± 4.15
P	165	9.22 ± 3.84	9.90 ± 4.22	11.04 ± 4.86
H F	145	11.09 ± 4.21	11.85 ± 4.51	12.92 ± 5.06
H N	28	11.05 ± 3.15	11.81 ± 3.35	13.01 ± 3.75
P H	27	9.75 ± 3.74	10.51 ± 3.97	11.72 ± 4.45
F N	9	11.63 ± 4.92	12.31 ± 5.05	13.33 ± 5.45
F P	68	10.46 ± 4.11	11.20 ± 4.45	12.26 ± 4.87
N P	5	10.46 ± 3.83	11.69 ± 3.74	13.69 ± 3.58
H F N	13	11.90 ± 4.17	12.67 ± 4.18	13.88 ± 4.27
H F P	154	9.86 ± 4.35	10.47 ± 4.66	11.45 ± 5.20
H N P	21	10.68 ± 4.28	11.67 ± 4.77	13.50 ± 5.85
F N P	5	12.75 ± 4.18	13.51 ± 4.28	14.88 ± 4.49
All types	27	10.88 ± 5.40	11.64 ± 5.72	12.80 ± 6.48

marked difference in foot temperatures both between and within flocks, as well as a great degree of temperature overlap, with no statistically significant difference between normal and abnormal feet. These factors make it difficult, if not impossible, to select a cut-off point for distinguishing between normal and abnormal using the approaches developed for this study. Therefore, the lesion-specific temperature differences could not be considered large enough to be of practical significance.

As hypothesised, hyperkeratotic regions were statistically significantly colder than normal regions. Papillomatous growths also appeared colder, rather than warmer, compared to normal regions. This could be due to epithelial proliferation and increased distance from the epithelial surface to underlying vasculature, similarly to hyperkeratosis. Although a thermal effect was detected with nodular lesions using general linear mixed effect modelling, areas with nodular lesions could be either colder or warmer than areas without, depending on the region in question. No effect was found related to fissures using either model. It is possible that ischaemia and vascular damage, as described in other species (Remple and Al-Ashbal 1993), in fact reduces cutaneous circulation and therefore also heat emission from affected sites, which may have contributed to some of the findings in this study. Alternatively, it can be questioned whether observed lesions are indeed all inflammatory, as expected, or whether a number of these lesions are instead inactive or healing. This is particularly the case with nodular lesions, which could sometimes be confused with large callouses or swellings associated with deep fissures. Wyss et al. (2014) discussed this phenomenon when using fine granular sand as a substrate, and hypothesised that some of the observed N2 lesions may in fact be healing fissures rather than true necrotic nodules. If this is true, one may expect to see a different thermal pattern between the healing lesions and true nodules with necrotic centres. Whilst Wyss et al. (2015) confirmed the presence of inflammation and neovascularisation on histopathology of papillomatous lesions, further research into the pathology associated with the other lesion types and their progression and healing is required.

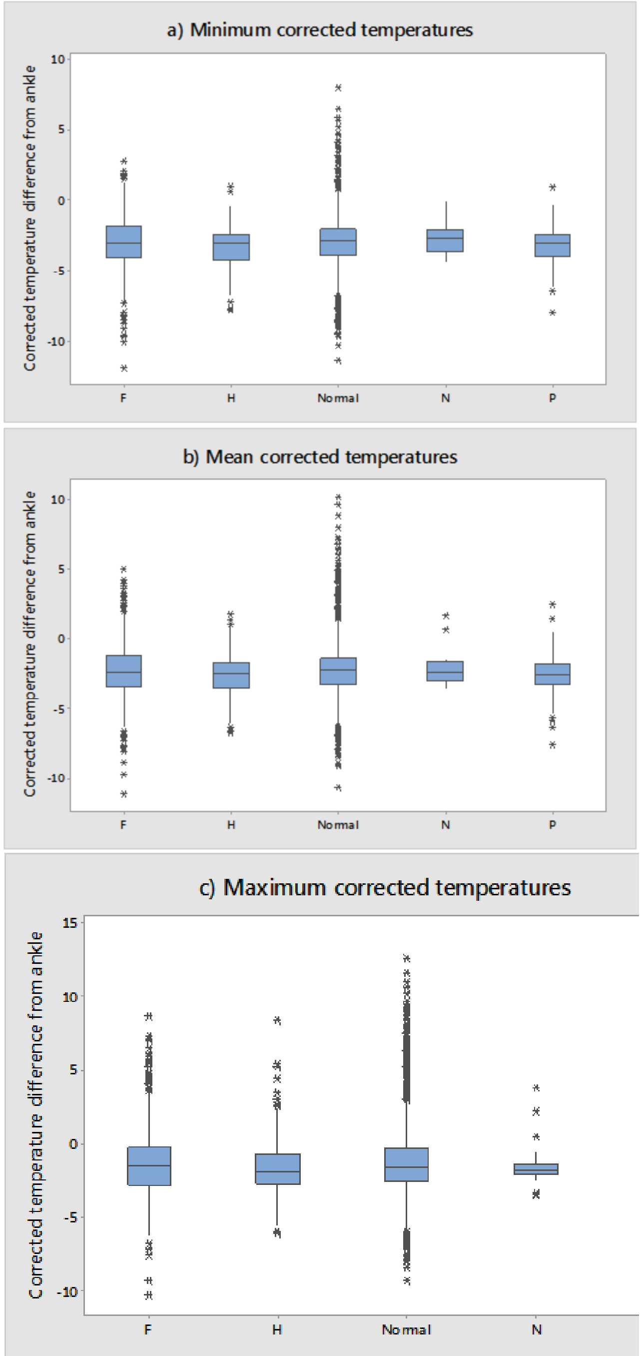

Figure 3. Boxplots showing the corrected temperature distributions for each lesion type against normal for a) minimum, b) mean and c) maximum values

The results of this study largely agree with those reported by Duncan et al. (2014) for penguins. However, this stands in stark contrast to the conclusion drawn for poultry by Wilcox et al. (2009), where IRT appeared to be a very promising diagnostic tool for pododermatitis. However, Wilcox et al. (2009) did not touch on the possible effects of ambient temperature or report on the range of foot temperatures seen, both important factors seen in flamingos and penguins. The omission of this information makes it difficult to compare findings between studies and may contribute to the different conclusions. Absolute temperature gradients between the metatarsal pad and distal third phalanx, as utilised in the poultry and penguin studies, would likely not be useful in populations with such large temperature differences as found in this study because cold feet will inevitably never achieve the same temperature gradients as would hot feet. However, it is possible that IRT may be of use in a uniformly controlled environment such as can be achieved in a poultry house, although these conditions would rarely be obtainable when dealing with wild or extensively managed zoo species.

The thermoregulatory adaptations of the flamingo also pose a particular challenge with regards to thermographic assessment of the feet. Firstly, the species possesses a very well-developed *rete tibiotarsale* and can effectively regulate blood circulation to the hind legs in order to help maintain an optimum core temperature in different environmental conditions (Midtgård 1980). This became apparent when observing the wide range of mean foot temperatures within the three flocks, as well as the highly significant difference in mean foot temperature distributions between the three flocks, which is most likely related to ambient temperature conditions at the time of sampling. These findings suggest that using absolute temperatures to assess for flamingo foot pathology is almost certainly meaningless. Likewise, it is also unlikely that comparing absolute temperatures within a foot over time will be meaningful in flamingos due to the factors mentioned above. Although this was not possible to confirm in this study due to lack of serial measurement, it has been observed to be the case with penguins, where individual animals showed significant foot temperature variations between samplings (Duncan et al. 2014).

Figure 4. Example thermal images and corresponding photographs of affected feet. Top: Feet affected by N2 lesions of the base with visible hot spots (lighter grey) on thermogram. Middle: Right foot showing a marked increase in heat emission from the medial (2nd) digit, extending up the medial aspect of the leg. The other digits are visibly colder (darker grey). The fissure is easily seen on the thermogram and is likely associated with active inflammation. Bottom: Feet affected by both N2 and F2 but without any significant hot spots. The thermogram shows a typical pattern observed in flamingos with a gradual heat dissipation from base to distal toes. The right foot and distal leg are warmer than the left, indicating that this leg has been recently rested.

Secondly, the flamingo's behavioural adaptation of resting on one leg, particularly in water, further complicates thermal image analysis. Medical thermographic assessment traditionally relies on the thermal symmetry of the subject, where the left and right sides of a healthy body are assumed to be of the same temperature so that the structure of interest can be compared to that on the contralateral side (Jones 1998). However, this approach is of less use when studying flamingo feet due to this behavioural adaptation, which means that the rested leg often is considerably warmer than the other. Each individual foot must therefore be assessed on its own, preferably by using a reference point within the same leg for normalisation. This technique was used in one study when IRT was assessed as a diagnostic tool in rheumatoid arthritis in human finger joints, where a region on the ipsilateral wrist was used as a reference point for the hand (Varjú et al. 2004). Similar corrections were attempted in this study by using an unaffected point on the ankle as a reference point for the foot, with the assumption that lesions that are warm due to inflammation should be proportionately warmer than the rest of the foot, regardless of the baseline temperature of the foot at the time of imaging. The ankle was chosen in this case as it is an area that is very rarely affected by lesions or obscured by other structures during imaging, thus serving as an easily accessible, unaffected reference point. However, normalising foot temperatures against the ankle may potentially wash out some signal as heat from inflammation is often visible as a gradient away from the affected site (Amezcua et al. 2014).

There are some inherent limitations in this study which could be addressed in future work. Firstly, as data collection was opportunistic as a part of scheduled annual catches, it was not possible to do a serial study where the relationship between lesion development and thermal image pattern could be explored. It is therefore impossible to say if some unexplained thermal variations in normal feet were due to random variation or subclinical lesions, or even due to other unrelated pathology within the leg or the foot. Secondly, for the same reason it was not possible to control the time the flamingos spent off the ground prior to thermal imaging, which has been shown to have a significant effect on thermal patterns in penguin feet (Duncan et al. 2014). Thirdly, the existing visual scoring system possesses some limitations. Most notably, scoring is at least partly subjective and influenced by the operator's own experiences and definitions, particularly in cases where lesions are borderline. To overcome some of this inherent variability, all photos in this study were scored by the first author and adjusted as closely as possible to the definitions set out by Nielsen et al. (2010). It is interesting to note that the overall lesion prevalence in this study was similar to the high prevalence reported by Nielsen et al. in 2010, albeit with some differences in regional distributions, which highlights the potential welfare implications associated with this condition. Nevertheless, the scoring system remains fairly subjective, particularly when photographs are used on their own, which may have influenced results when comparing against an objective tool such as thermography.

Although the methods employed in this study failed to yield results that can be used for diagnostic purposes, IRT may still offer some additional information to clinical examination in individual cases. Although not supported by the statistical analyses used here on pooled samples, some images showed clear hot spots over lesions that would normally be expected to be associated with inflammation based on visual appearance, suggesting that studying individual thermograms may still have some value;

although they must be interpreted with great care. Figure 4 (top and middle images) shows two such examples, where a subjective assessment of overall thermal patterns may reveal more information than visual inspection alone, and where IRT appears to aid in distinguishing between active and inactive lesions on a case-by-case basis. By contrast, in the bottom image, no obvious abnormal thermal pattern is visible for the same lesion types. There is currently insufficient evidence available to determine if such subjective assessment can offer more information than clinical examination alone and be useful for the management of pododermatitis in flamingos in a clinical setting. The use of different approaches to determine if IRT is at all viable in a diagnostic setting for this species would therefore be beneficial.

Further in-depth studies into the pathogenesis of foot lesions in flamingos, alternative thermal image analysis techniques, as well as the use of IRT in other species, would be useful to look for supportive evidence for the clinical usefulness of thermography for this condition, and to better establish the relationship between disease progression and changes in thermal signatures.

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