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Hot dogs: Thermography in the assessment of stress in dogs (Canis familiaris)-A pilot study

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Hot dogs: Thermography in the assessment of stress in dogs (Canis familiaris)-A pilot study / Travain, Tiziano; Colombo, Elisa Silvia; Heinzl, Eugenio; Bellucci, Danilo; Prato Previde, Emanuela; Valsecchi, Paola Maria. - In: JOURNAL OF VETERINARY BEHAVIOR. - ISSN 1558-7878. - 10:1(2015), pp. 17-23. [10.1016/j.jveb.2014.11.003]

Availability: This version is available at: 11381/2798927 since: 2015-12-16T15:19:12Z

Publisher: Elsevier USA

Published DOI:10.1016/j.jveb.2014.11.003

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note finali coverpage

(Article begins on next page)

Elsevier Editorial System(tm) for Journal of Veterinary Behavior: Clinical Applications and Research Manuscript Draft

Manuscript Number:

Title: Hot dogs: thermography in the assessment of stress in dogs (Canis familiaris) - A pilot study

Article Type: Original Research Papers (Regular Paper)

Keywords: Dog; Stress; Veterinary practice; Behavior; Infrared thermography

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Highlights

- We applied IRT technique to measure stress response in dogs.
- Eye temperature and rectal temperature positively correlate in dogs.
- IRT detects eye temperature changes in dogs during stress situations.



UNIVERSITÀ DEGLI STUDI DI PARMA

DIPARTIMENTO DI NEUROSCIENZE Unità di Biologia del Comportamento Area delle Scienze 11/A 43124 Parma www.unipr.it

Prof. Karen Overall School of Medicine University of Pennsylvania Glen Mills, PA USA

May 22nd, 2014

Dear prof. Overall,

on behalf of myself and all co-authors, please find enclosed our manuscript entitled "Hot dogs: thermography in the assessment of stress in dogs (*Canis familiaris*) – A pilot study". This study represents the first application in dogs of a non-invasive technique (infrared thermographic camera) to assess eye temperature during a stressful situation and it is of potential interest for researchers involved in developing experimental protocols aimed at enhancing animal welfare.

We would like to submit it to the Journal of Veterinary Behavior: Clinical Applications and Research for publication. The manuscript has not been submitted elsewhere and guidelines for use of animals in research have been followed.

Thank you for your kind attention.

Yours sincerely,

Rias trach

Tiziano Travain

Dipartimento di Neuroscienze Unità di Biologia del Comportamento Università degli Studi di Parma Parco Area delle Scienze 11/A 43124 Parma - Italy Fax: 0039 0521 905657 e-mail: tiziano.travain@studenti.unipr.it The *Journal of Veterinary Behavior: Clinical Applications and Research* encourages submission of multi-author papers and those with acknowledgments that accurately reflect help received in the preparation of the manuscript or in the research and analysis.

Because of ethical issues raised by recent scientific debate, we wish for the authors to note that we do not accept papers that have "courtesy" authorships, nor those where acknowledgments do not accurately reflect contributions. The following are not acceptable:

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I am the senior author and understand and will comply with the above policy.

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Date: May 22, 2014

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23 Abstract

24 Infrared thermography (IRT) represents a novel, non-invasive method to investigate stress responses in 25 animals. Despite the large existing bibliography about stress responses in dogs, the potential use of IRT in 26 assessing dogs' stress reactions has not been investigated so far. This study evaluates the usefulness of IRT to 27 assess dogs' emotional responses to an unpleasant and stressful event. Following a preliminary test, aimed to 28 evaluate the correlation between eye-temperature and rectal temperature in dogs in a stressful situation, a sample 29 of 14 adult healthy dogs was observed during a standardised veterinary examination, carried out by an unfamiliar 30 veterinarian in the presence of their owner. Dogs' behavior and eye temperature were recorded before the start of 31 the veterinary visit, during and after the clinical examination. Dogs' level of activity and stress related behaviors 32 varied across the different phases of the visit; interestingly, dogs showed an increase in eye temperature during 33 the examination phase, compared to both pre-examination and post examination phases, despite they had a 34 significant decrease in their level of activity. However, it also emerged that the thermographic camera, although 35 remote and non-invasive, was at some extent disturbing for dogs, as they showed avoidance behaviors, averting 36 gaze and/or turning their head, exclusively when the thermographic camera was oriented to them. Overall results 37 suggest that IRT may represent a useful tool to investigate emotional psychogenic stress in dogs. Nevertheless 38 further research is needed to establish the specificity and sensivity of IRT in this context and to assess 39 how different dogs' characteristics, breed, previous experience and the nature and severity of the 40 stressor could influence the magnitude and type of the stress response.

41

42 *Keywords:* Dog; Stress; Veterinary practice; Behavior; Infrared thermography

43 **1. Introduction**

44 Infrared thermography (IRT) is a passive, remote and non-invasive method that measures surface temperature, 45 detecting infrared radiation emitted by a subject and providing a pictorial representation of body temperature in 46 animals (Speakman and Ward, 1998; Stewart et al., 2005). Several studies have shown that IRT provides 47 information on an animal's health: it allows detecting inflammatory conditions and infections (Berry et al., 2003; 48 Fonseca et al., 2006; Schwartzkopf-Genswein and Stookey, 1997) and represents a useful method to assess acute 49 and chronic stress in laboratory (e.g. Ludwig et al., 2007) and farm animals (e.g. Stewart et al., 2007, 2008). In 50 fact, there is evidence of a close relationship between stress and the metabolic system: when an animal becomes 51 stressed the HPA axis is activated and, as a result of increases in catecholamines and cortisol levels as well as 52 blood flow responses, it will produce changes in the animal's heat production and loss (Schaefer et al., 2002). A 53 condition known as "stress-induced hyperthermia" (SIH), or a relative short-lasting rise in core body temperature 54 induced by stress, have been reported in rodents, baboons, sheep, impalas, chimpanzees and humans (Cook et 55 al., 2001; Groenink et al., 1994; Olivier et al., 2003; Vinkers et al., 2010).

56

57 A number of studies based on IRT have shown that the temperature of the eye is a good indicator of 58 stress (e.g. Cook et al., 2001, 2006; Pavlidis et al., 2002). In particular, the temperature of small areas around the 59 posterior border of the eyelid and the lacrimal caruncle, which have rich capillary beds innervated by the 60 sympathetic system, respond to changes in blood flow (Pavlidis et al. 2002; Stewart, 2008). The lachrymal 61 caruncle is an anatomical area very sensitive to both pain and stress events affecting an individual, and changes 62 in its temperature have been attributed both to the sympathetic response of the autonomic nervous system (ANS) 63 reaction and to HPA activation (Cook et al., 2001; Stewart, 2008; Valera et al., 2012). The sympathetic branch of 64 the ANS responds rapidly, preparing the individual for the "fight or flight" reaction (Cannon, 1929; Koolhaas et 65 al., 2010), whereas the parasympathetic system is predominant during passive reactions such as freezing (Alm, 66 2004; Romero, 2010); the HPA axis activation is more delayed and is particularly sensitive to psychogenic 67 stressors not producing physical damage (Gabry et al., 2003; Johnson et al., 1996; Toates, 2001). Stewart and 68 colleagues (2007, 2008) found that in cattle, during the first few seconds of a stressor presentation (acute phase),

69 eye temperature dropped rapidly, likely because of a sympathetic response (peripheral vasoconstriction); 70 however, if the stressor persists for a longer time, the HPA axis induces a cortisol release, that can be maintained 71 from minutes to hours (chronic phase), causing several thermogenic reactions in tissue metabolism (Valera et al., 72 2012). Therefore, HPA axis response to stressors, along with peripheral vasodilatation due to the 73 parasympathetic activation that follows the initial sympathetic response, can produce an increase in eye 74 temperature (Cook et al., 2001; Valera et al., 2012). Recently Johnson and colleagues (2011) found a good 75 correlation between thermographic eve temperature and rectal temperature in ponies, suggesting that eve 76 temperature can be a valid index to core body temperature and its variation, and could be useful also to detect 77 stress-induced-hyperthermia in animals. Similarly, Vianna and Carrive (2005) reported that in fear-conditioned 78 rats' eye and body temperature increased. There is also some evidence indicating that eye temperature increases 79 when there is a psychological component of stress, such as in response to the anticipation of catheterization 80 procedure in cows (Stewart et al., 2007) and lying in humans (Pavlidis et al., 2002).

81

82 Dogs are widespread companion animals that highly depend on humans for both health and care, and 83 several studies have investigated stress responses in dogs in a variety of situations, using both behavioral and 84 physiological variables (Beerda et al., 1997, 1998; Fallani et al., 2007; Hennessy et al., 2013; Palestrini et al., 85 2005). However, as far as we know, IRT has been considered as a diagnostic technique in dogs only recently 86 (Biondi et al., 2013), and it has never been used to investigate dogs' stress reactions so far. Veterinary 87 examination has been reported to be stressful for most dogs (Döring et al., 2009), with dogs exhibiting fear 88 reactions especially during the clinical examination, but also showing anticipatory fear reactions prior to entering 89 the veterinary clinic for being examined (Stanford, 1981). The aim of the present study was to evaluate the 90 potentialities of infra-red thermography in the investigation of dogs' psychological stress in veterinary practice. 91 After a pre-test carried out on a sample of dogs and aimed to evaluate the correlation between eye-temperature 92 and rectal temperature in dogs, a different sample of dogs was tested during a standardised veterinary 93 examination, carried out by an unfamiliar veterinarian in the presence of their owner, and their behavior and eye 94 temperature variations were recorded before the visit, during and after the clinical examination. The examination

aim was to induce a negative psychological state in dogs, and thus it consisted of routine and not painful
assessments of dogs' health.

97

98 **2. Pre-test**

99 There is evidence of a relationship between thermographic eye temperature and body temperature and its 100 variation, and it has been suggested that eye temperature could represent an useful tool to assess stress in animals 101 (Johnson et al., 2011; Vianna and Carrive, 2005): however, to our knowledge, there are not studies on dogs 102 stress responses based on IRT; thus, in the present pre-test whether a correlation between eye-temperature and 103 rectal temperature exists also in dogs.

104

105 2.1 Materials and methods

106 2.1.1 Subjects

107 The subjects were 20 healthy dogs (eight females, 12 males) of different breeds and body size, whose 108 ages ranged from 7 months to 15 years (mean = 9.0 years, SD = 4.67 years). The sample included 16 pure-breed 109 dogs (one Pinscher toy size, one Pug, two Dachshund miniature size, one West Highland White Terrier, two Jack 110 Russel Terrier, three Poodles medium size, one Golden Retriever, one Czechoslovakian Wolfdog, one Italian 111 Pointer, one Cane Corso, one Spanish Galgo, one English Setter) and four medium size mixed-breed dogs. All 112 the dogs were kept for companionship and lived within the human household. They were accustomed to being 113 taken to the veterinary; none of them was reported to be aggressive during veterinary examinations. All the 114 owners were informed about the aims of the study and the procedure, and their informed consent was obtained. 115 None of these dogs took part in the subsequent test. 116 117 2.1.2 Procedure

The study was conducted in a veterinary clinic in Milan, Italy (D.B. Veterinary Clinic). The dogs were visited in opening hours, during a routine booster vaccination. The pre-test consisted of a unique phase in which dogs' rectal and eye temperature were measured.

121

122 The owner and the dog went into the examination room, the veterinarian lifted the dog on the 123 examination table and measured rectal temperature. Dogs were not physically restrained, so the owner assisted 124 the vet holding his dog if necessary, preventing it from jumping down from the table and calming it as needed. 125 The entire examination lasted 2 minutes. The sequence of events was standardised and the examination 126 circumstances (handling, room-features, equipment used) were always the same.

127

128 2.1.3 Data collection

129 The thermographic infrared images were captured by a certified technician (E.H.) using a portable IRT 130 camera (AVIO TVS500® camera, NEC, Japan) with standard optic system, and analyzed with IRTAnalyzer 131 Software® (Grayess, FL, USA). To calibrate the camera reflectivity temperature, samples were taken and 132 emissivity was set at 0.97. Several images per dog were collected during each of the 3 phases of the experiment, 133 to select the images that provided the most optimal operating conditions for analysis (90 $^{\circ}$ angle and 1 m of 134 distance). A total of 62 (per dog: mean \pm SE = 3.10 \pm 0.18; minimum = 2; maximum = 4) images were analyzed 135 evaluating the emission of eyes' lachrymal sites. The maximum temperature for each lachrymal site was 136 determined using an Instantaneous Field of View of 1.68 mm at 1 m of distance, within an oval area traced 137 around the eye, including the eyeball and approximately 1 cm surrounding the outside of the eyelids. Only 138 images perfectly on focus were used (Fig. 1). To optimize the accuracy of the thermographic image and to 139 reduce sources of noise, before every work session the same image of a Lambert surface was taken to define the 140 radiance emission and to nullify the effect of sunlight or other surface reflections on tested animals. Using this 141 method, it is possible to control for room temperature and for other external artefacts.

142

Rectal temperature was taken by D.B. with a normal veterinary rectal thermometer.

143

144 2.1.4 Statistical analysis

145 To assess if there is a correlation between mean rectal temperature and mean eye temperature, given 146 normal distribution and number of subjects of the sample, Pearson correlation was calculated.

147

148 2.2 Results

The mean rectal temperature of the sample of dogs was 38.57 °C (SD = 0.43 °C), while the mean eye temperature was 36.18 °C (SD = 0,68 °C). Pearson correlation between eye and rectal temperature was r = 0.661, p = 0.002.

152

153 2.3 Discussion

154 This test aimed at assessing the existence of a correlation between eye temperature measured with 155 infrared thermography (IRT) and rectal temperature in dogs. To reach this goal we took infrared pictures while 156 rectal temperature was taken during a short standardized veterinary visit. 157 Johnson and colleagues (2011) found a correlation between thermographic eye temperature and rectal 158 temperature in ponies: further evidence that eye temperature can be a valid index to core body temperature and 159 its variation comes from Schaefer et al.'s study (2007), showing that infrared thermography was able to identify 160 calves at early stages of illness, and from Dunbar et al.'s work (2009), reporting no significant differences in 161 body temperature and eye temperatures of well-focused thermograms in mule deers.

Our data are in line with previous findings in the literature, and indicate that eye temperature is a good
indicator of internal temperature also in dogs: therefore we used it for the following test.

164

165 **3. Test**

166

167 3.1 Subjects

The subjects were 14 adult and clinically healthy dogs (nine females, five males) of different body size, whose ages ranged from 1.5 to 11 years (mean = 5.8 years, SD = 2.54 years). The sample included nine purebreed dogs (one Poodle toy size, two Fox Terrier, two Jack Russell Terrier, one Dachshund miniature size, one Golden Retriever, one Bergamasco Shepherd and one Great Anglo-French Hound) and five mixed-breed (two small-medium, one medium, one Alaskan Malamute mix and one Siberian Husky mix). All the dogs were kept

173	for companionship and lived within the human household. All dogs had previous experience of being taken to
174	the veterinary and, as reported by their owners, they clearly disliked this kind of situation; however, none of
175	them was reported to be aggressive during veterinary examinations. All the owners were informed about the
176	aims of the study and the procedure, and their informed consent was obtained.
177	
178	3.2 Procedure
179	The study was conducted in the same location and under the same condition as the pre-test. The
180	procedure consisted of three consecutive phases in which dogs' behavior was recorded and eye temperature was
181	measured.
182	
183	Phase 1, pre-examination. After the dog and the owner entered the veterinary clinic waiting room, the
184	owner was asked to sit quietly keeping the dog on leash next to him for 10 minutes, pretending to wait his turn.
185	
186	Phase 2, examination. This phase was a standardised general examination. The owner and the dog went
187	into the examination room, the veterinarian lifted the dog on the examination table and performed the following
188	checks: conjunctiva, ears and oral mucosa, palpation of the dog's abdomen, examination of lymph nodes and
189	heart auscultation with a stethoscope. Dogs were not physically restrained, but the owner assisted the vet holding
190	the dog if necessary, thus preventing his/her dog from jumping down the table and calming it. The entire
191	examination lasted between 4 to 5 minutes (average duration = $262.22 \text{ s} \pm 17.86 \text{ SE}$). During this phase the
192	
	sequence of events was standardised and the examination circumstances (handling, room-features, equipment
193	sequence of events was standardised and the examination circumstances (handling, room-features, equipment used) were always the same.
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193 194 195 196 197	sequence of events was standardised and the examination circumstances (handling, room-features, equipment used) were always the same. Phase 3, <i>post examination</i> : the veterinarian put the dog down to the ground and sat at his desk with the owner, talking about the dog for 5 minutes. Then the dog and the owner left the examination room and the clinic.

198 *3.3 Data collection*

199 The thermographic infrared images were captured following the same procedures used in the pre-test 200 (Fig. 1). A total of 546 (per dog: mean \pm SE = 39.00 \pm 4.20; minimum = 17; maximum = 66) images were 201 analyzed evaluating the emission of eyes' lachrymal sites.

202 Dogs' behavior during the three different phases was recorded using a camcorder (Leica Dicomar, 203 Panasonic, Japan) placed on tripod and behavioral data were scored from videos using Solomon Coder beta® 204 12.09.04 (ELTE TTK, Hungary). The ethogram consisted of two main categories of mutually exclusive 205 behaviors, and in particular we focused on: 1) dogs' level of activity, namely whether the subject was engaged in 206 observable physical activity, like walking, jumping or being agitated while standing on the table (dynamic), or 207 was sitting, standing or lying down and, therefore, movement was almost absent or very limited, i.e. head or ears 208 movements (static); 2) stress/fear related signals which included: shake off, yawning, nose/lip-licking, paw-lift, 209 mouth opening/closing, panting, posture change, freezing and avoidance (see Table 1 for a description) (Beerda 210 et al., 1997, 1998). In addition, to evaluate whether the mere fact of directing the thermographic camera 211 towards the dogs' muzzle could affect their stress displays, during coding each behavioral element was 212 accompanied by a marker indicating the presence of the thermographic camera. The dogs' behavior was coded 213 from videos by one of the authors (E.S.C) whereas a second independent coder (T.T.) analyzed 20% of the data: 214 Spearman correlations were calculated for the main behaviors (dynamic: r = 1, P < 0.001; freezing: r = 1, P < 0.001; free 215 0.001; panting: r < 1, P = 0.001; avoidance: r = 0.95, P = 0.004; stress/fear signals: r = 0.986, P < 0.001; as 216 pointed out in Table 1, stress/fear signals were pooled for purpose of analysis).

217

218 2.4 Statistical analysis

Differences in dogs' degree of activity, stress/fear related behaviors and eye temperature in the three phases were evaluated using non-parametric and two-tailed statistical tests, setting alpha at 0.05. Stress/fear signals reported in Table 1 were pooled due to their low occurrence, with the exception of avoidance, which occurred only when the thermographic camera was oriented to the dogs' muzzle, and of panting and freezing, which were measured as duration. Friedman's ANOVA for ranks with the minimun difference post hoc tests (Siegel and Castellan, 1988) were run to detect differences among phases in dogs' eye temperature and to

- 225 evaluate differences among phases in behavior and stress/fear related signals. Wilcoxon rank-sum test was used
- to evaluate whether the presence of the thermographic camera affected dogs' behavior. All the statistical

analyses were carried out with SPSS Statistics 21 (IBM, NY, USA).

228

4. Results

230 4.1 Termographic data

Dogs' eye temperature increased during the examination phase and decreased to pre-examination values in the post examination phase (Fig. 2). The Friedman's ANOVA showed that the variation in eye temperature was significant ($\chi^2 = 8.714$; df = 2; *P* = 0.013), and the post-hoc test revealed a significant difference between pre-examination and examination phase (minimum D = 0.726; D = 0.929, *P* = 0.05), and between examination and post examination phase (minimum D = 0.968, D = 1.000, *P* = 0.01).

236

237 4.2 Behavioral data

238

239 4.2.1 Comparison of the three phases

Considering the level of activity, there was a significant difference among the three different phases in the duration of dynamic behavior (Friedman: $\chi^2 = 16.000$; df = 2; *P* < 0.001): in particular, dogs' activity significantly decreased in the examination phase (pre examination vs. examination: minimum D = 0.968, D = 1.143, *P* = 0.01; examination vs. post examination: minimum D = 0.968, D = 1.429, *P* = 0.01; Fig. 2). Dogs showed stress/fear signals during the whole test (on average 2.40 stress/fear signal per min), with some signals occurring more than others (Table 2).

246

The highest frequency of stress signals, apart from freezing, was observed in Phase 3 (post examination: 248 2.78 signals/min), whereas the lowest one during Phase 2 (examination phase: 1.68 signals/min). Overall mouth 249 opening/closing was the most frequent signal of stress (0.78 times/min during the whole test), whereas yawning 250 and shaking off were the two less frequent signals (both 0.10 times/min). Differences in stress/fear signals

frequency across phases were not statistically significant (Friedman: $\chi^2 = 4.000$; df = 2; P = 0.135), except for 251 252 avoidance behavior which varied significantly among phases: it dropped during the examination phase, 253 increasing again in the post examination phase (Friedman: $\chi^2 = 7.091$; df = 2; P = 0.029). Nine dogs (64.3%) 254 showed panting during the procedure and this behavior occurred for 22.2% of the overall time, being present in 255 Phases 1, 2 and 3 on average for 23.8%, 14.1% and 26.1% of the time respectively. Although panting decreased 256 during the examination phase, differences in this behavior among phases were not significant (Friedman: χ^2 = 257 5.314; df = 2; P = 0.07). Freezing behavior was found exclusively during the examination phase, and seven of 258 the 14 dogs (50% of the subjects) exhibited this behavior: this may explain why during the examination phase 259 there was the lowest frequency of stress/fear signals and the lowest duration of panting and dynamic behavior.

260

261 4.2.2. Effect of thermographic camera

It emerged that throughout the test dogs showed avoidance behavior, i.e. turning the head and/or looking away, exclusively when the technician oriented the thermal camera towards the dogs' muzzle to capture images (Fig. 3). Thus, a further analysis was carried out to assess differences in the frequency of dogs' other stress/fear signals and the duration of freezing and panting behaviors when the thermographic camera was directed vs. not directed towards the dogs' muzzle. Results revealed a significant difference in the duration of freezing (Wilcoxon: Z = -2.197; P = 0.028), a trend in frequency of stress/fear signals (Wilcoxon: Z = -1.726; P = 0.084) and no significant difference in the duration of panting (Wilcoxon: Z = -1.244; P = 0.214).

269

270 **5. Discussion**

In the current study we aimed to assess whether infrared thermography (IRT) would be a useful tool to assess stress conditions in dogs. As IRT has not been previously used on dogs to evaluate stress we first assessed the relationship between eye and body temperature with a test to verify if there is positive correlation between them. Results of the pre-test support previous findings (Johnson et al., 2011; Vianna and Carrive, 2005) indicating a relationship between eye temperature and body temperature in dogs. Then we exposed dogs to a common stressful situation, i.e. the veterinary visit (Döring et al., 2009), and recorded the behavioral signs of 277 stress showed by all the dogs and their eye temperature (i.e. the temperature of small areas around the posterior 278 border of the evelid and the lacrimal caruncle). Thermographic data revealed the presence of a peak in dogs' eve 279 temperature during the clinical examination phase. Since at the behavioral level this phase was characterized by 280 a clear drop in dynamic behavior and by the occurrence of freezing behavior in half of the subjects, it is unlikely 281 that the observed increase in eye temperature simply depended on dogs' activity. Rather, it appears that the 282 temperature increase was associated to a condition of stress due to the visit itself. In this respect our results 283 confirm those by Döring and colleagues (2009), who found that veterinary examination is perceived as stressful 284 by dogs. Our procedure did not involve any physical injury to animals, and thus it likely represented a 285 psychogenic stressor, due to exposure to a novel and threatening environment, unpredictability and lack of 286 control over external events (Hennessy, 2013; Toates, 2001). Probably, when faced with the veterinarian, dogs 287 realized that no active strategy was possible and showed a passive behavior, displaying a more static posture 288 than in the other conditions or even showed freezing. HPA axis is especially sensitive to this kind of 289 psychogenic stressor and its effects on metabolism, along with peripheral vasodilatation due to parasympathetic 290 activation during freezing response (Alm, 2004; Romero, 2010), may explain the increase in eye temperature 291 detected by thermography when dogs were on the examination table (Cook et al., 2001; Valera et al., 2012). 292 Moreover, all our dogs had previous experiences with veterinary examinations, so the anticipation of an 293 unpleasant experience could have played a role in the stress response, supporting studies that have linked a 294 cognitive component of stress with the increase in eye temperature (Pavlidis, 2002; Stewart et al., 2007; Valera 295 et al., 2012; Vianna and Carrive, 2005). This is the first study using IRT to assess dogs' responses to an 296 unpleasant situation and, as there is evidence that factors like breed, age and previous experience could influence 297 the magnitude and type of the stress responses (Clark et al., 1997; D'Eath et al., 2010; Valera et al., 2012), 298 further studies should be undertaken to highlight the potential role of these variables on eye temperature and 299 other stress responses in dogs. In particular, it would be interesting to compare dogs with and without previous 300 experience with veterinary examinations to investigate to what extent anticipation of an unpleasant experience 301 could have modulated the dogs' stress response and eye temperature.

302 It is worth noting that dogs also exhibited an avoidance reaction only when the thermographic camera 303 was directly oriented towards their muzzle. In particular, this reaction decreased during the examination phase 304 and increased again in the post examination phase, exceeding pre examination phase level. Furthermore, freezing 305 and other stress/fear signals increased when the thermographic camera was directed toward the dogs, and this 306 suggests that, although remote and non-invasive, it was to some extent disturbing for dogs and that possibly it 307 determined a short-term sensitisation effect. It is also possible that dogs' avoidance response depended also, at 308 least in part, on having an unfamiliar human facing them while holding a strange object. There is evidence that 309 for most non-human species a direct and prolonged duration of gaze is considered as a threatening behavior 310 (Emery, 2000), and this has been reported also for wolves and dogs (Bradshaw and Nott, 1995; Schenkel, 1967). 311 It has also been shown that, in the absence of other accompanying signals, a direct and prolonged gaze puts dogs 312 in an uncomfortable situation (Gácsi et al., 2013; Hernádi et al., 2012; Vas et al., 2005, 2008). Thus, it is 313 possible that dogs perceived a prolonged photo framing by a static and silent human as a threat and thus reacted 314 with avoidance, averting gaze and turning the head.

315

316 6. Conclusions

317 This study represents a first step in the validation of infrared thermography as a method for 318 measuring stress in dogs. Our results indicate that the IRT is a useful tool to detect temperature 319 variation due to emotional stress in dogs. Further researches are needed to establish whether sensivity 320 of IRT in context-dependent, i.e. emotional distress, or is applicable in positive context as well. The 321 fact that dogs showed avoidance behaviors, looking away or even turning their head, when the 322 thermographic camera was focused on them, suggests that it could be a mild stressor per se, and this 323 aspect deserves further investigation. Furthermore, given the small sample size of this pilot study it is 324 important to assess how different dogs' characteristics, i.e. breed, age, previous experience, and the 325 nature and severity of the stressor could influence the magnitude and type of the stress response and 326 consequently the applicability of the IRT. It would be also interesting to compare IRT with other

327	physiological	measures of stress.	such as heart	rate variability.	in order to	better understand the
211	physiological	measures or scress,	Such as near	race farmering	,	oottor anaorotana mo

328 physiological mechanisms that cause changes in dogs' eye temperature.

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330 Acknowledgements

331 We thank all the dog owners who kindly participated in this study. Tiziano Travain and Elisa Silvia

332 Colombo were supported by a doctoral grant from the Università di Parma and Università di Milano

respectively. Partial funding was from Università di Parma (FIL 2012 to P.V.).

334

335 Ethics Statement

336 No special permission for use of animals (dogs) in non-invasive observational studies is required in Italy.

337 The relevant ethical committee is the Ethical Committee of the Università degli Studi di Milano. All dog owners

were informed about the nature and scope of the study and their written consent was obtained before the studywas initiated.

340

341 **Conflict of interest**

None of the authors of this paper has a financial, personal or other relationship with other people or organizations within three years of beginning this work that could inappropriately influence or bias the content of the paper.

345

346 Authorship Statement

- The idea for the paper was conceived by Tiziano Travain, Elisa Silvia Colombo, Emanuela Prato Previde
 and Paola Valsecchi.
- 349 The experiments were designed by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl, Danilo

350 Bellucci, Emanuela Prato Previde and Paola Valsecchi.

351 The experiments were performed by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl and Danilo

352 Bellucci.

- 353 The data were analyzed by Tiziano Travain, Elisa Silvia Colombo and Eugenio Heinzl.
- The paper was written by Tiziano Travain, Elisa Silvia Colombo, Eugenio Heinzl, Emanuela Prato
 Previde and Paola Valsecchi.
- 356

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Table 1

Description and measure of mutually exclusive stress/fear related behaviors.

Behavior	Description	Frequency/Duration	
Panting ^a	Rapid breaths in short gasps	D (% on total time)	
Freezing ^a	Complete motionless, without noticeable panting	D (% on total time)	
Avoidance ^a	Lateral movement of the head and gaze averting	F (event/min)	
Nose/lip licking	Rapid extension and flicking of the tongue on the nose	F (event/min)	
	or between the lips		
Paw lift	Raise a paw at a time when it is standing or sitting still	F (event/min)	
Posture change	ure change Change posture, from lying on the ground to sitting or		
	standing and vice versa		
Mouth opening/closing	Rapid movements of opening/closing mouth	F (event/min)	
Shake off	Rapid movements of body shaking	F (event/min)	
Yawning	Involuntary intake of breath through a wide open		
	mouth, not for thermal regulation		

^a Behaviors singly analyzed, all other behaviors were pooled for purpose of analysis.

Table 2

Mean and SE of stress/fear related behaviors in the three phases of the experiment (pre examination, examination and post examination).

	Pre examination	Examination	Post examination	D 17-1	
Benavior	mean ± SE mean ± SE		mean ± SE	P Value	
Panting ^a	23.8 ± 7.1%	$14.1 \pm 6.9\%$	26.1 ± 7.7%	P = 0.07	
Freezing ^a	$0.0 \pm 0.0\%$	$11.3 \pm 4.4\%$	$0.0\% \pm 0.0\%$	Not analyzed ^b	
Avoidance ^a	0.16 ± 0.04	0.13 ± 0.06	0.60 ± 0.17	<i>P</i> = 0.029	
Nose/lips licking	0.68 ± 0.15	0.38 ± 0.15	0.63 ± 0.15		
Paw raising	0.09 ± 0.04	0.37 ± 0.18	0.11 ± 0.09		
Posture change	0.47 ± 0.08	0.12 ± 0.07	0.56 ± 0.16	<i>P</i> = 0.135	
Mouth open/close	0.88 ± 0.29	0.70 ± 0.22	0.58 ± 0.22		
Body Shaking	0.09 ± 0.03	0.00 ± 0.00	0.19 ± 0.04		
Yawning	0.14 ± 0.04	0.00 ± 0.00	0.11 ± 0.05		

^a Behaviors singly analyzed, all other behaviors were pooled for purpose of analysis.

^b Freezing behavior was not statistical analyzed because of it occurred only in examination phase.





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Figure captions

Fig. 1. Thermographic image and corresponding picture of Tika, Siberian Husky mix, in the waiting room (Phase 1).

Fig. 2. Eye temperature (°C) and mean percentage of dynamic behavior expressed by the 14 dogs during the three phases of the experiment (pre examination, examination, post examination).

Fig. 3. Mean frequency of avoidance and other stress/fear signals when the thermographic camera was oriented (with camera) or not to the dogs' muzzle (without camera) in the three phases of the experiment (pre examination, examination, post examination).