Physiological, physical and behavioural changes in dogs (*Canis familiaris*) when kennelled: Testing the validity of stress parameters

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HIGHLIGHTS

- A range of stress parameters were compared within-dogs at home and in kennels.
- Baseline values reflecting good dog welfare are presented for each parameter.
- Dogs were generally more active in kennels but showed large individual variability.
- Cortisol, VMA and surface temperature offer robust measures of canine arousal.
- Short-term kennelling did not seem to represent a negative stressor for these dogs.

ARTICLE INFO

ABSTRACT

Domestic dogs (*Canis familiaris*) housed in kennelling establishments are considered at risk of suffering poor welfare. Previous research supporting this hypothesis has typically used cortisol:creatinine ratios (C/Cr) to measure acute and chronic stress in kennelled dogs. However, the value of C/Cr as a welfare indicator has been questioned. This study aimed to test the validity of a range of physiological, physical and behavioural welfare indicators and to establish baseline values reflecting good dog welfare. Measurements were taken from 29 privately-owned dogs (14 males, 15 females), ranging in age and breed, in their own home and in a boarding kennel environment, following a within-subjects, counterbalanced design. Pairwise comparisons revealed that C/Cr and vanillylmandelic acid:creatinine ratios (VMA/Cr) were higher in the kennel than home environment ($P = 0.003$; $P = 0.01$, respectively) and were not associated with differences in movement/exercise between environments. Dogs' surface temperature was lower in kennels ($P = 0.001$) and was not associated with ambient temperature. No association with age, or effects of kennel establishment, kennelling experience, sex or source were found. Dogs were generally more active in kennels, but showed considerable individual variability. C/Cr and 5-HIAA:creatinine ratios (5-HIAA/Cr) were negatively correlated with lip licking in kennels. Baseline values for each parameter are presented. The emotional valence of responses was ambiguous and no definitive evidence was found to suggest that dogs were negatively stressed by kennelling. It was concluded that C/Cr and, particularly, VMA/Cr and surface temperature provide robust indicators of psychological arousal in dogs, while spontaneous behaviour might be better used to facilitate interpretation of physiological and physical data on an individual level.

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1. Introduction

Despite our historic relationship with domestic dogs (*Canis familiaris*), today, many council-funded animal shelters and charitable rehoming centres across the United States (U.S.) and United Kingdom (U.K.) are often filled to capacity with stray, abandoned and unwanted dogs [1,2]. The welfare of kennelled dogs is of concern, given that many experience minimal social contact, exercise and control over their environment [3] as well as unpredictable and high levels of noise, novelty and disrupted routines [4]. Such concern need not only be directed towards dogs in rehoming centres, but also to kennelled working dogs [3,5] and dogs kennelled for research purposes [6].

Previous research suggests that dogs experience acute stress following admission to kennels [5,7] and chronic stress in response to prolonged kennelling [6]. Stress "implies a threat to which the

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body needs to adjust", resulting in physiological and behavioural changes [8, p.E260]. For example, cortisol, which is secreted following activation of one of the major stress response systems – the hypothalamic–pituitary–adrenal (HPA) axis – [8], was found in significantly higher concentrations after one night in kennels than baseline levels measured both within- [5] and between-subjects in a home environment [7,9].

Urinary cortisol:creatinine ratio (C/Cr) is perhaps the most widely used physiological indicator reported in published studies of canine welfare [10], and is considered a valid measure of both acute [5,11] and chronic stress in dogs [6,12]. However, recent research has found C/Cr to be less reliable and less informative than previously thought for kennelled dogs [13]. Individual variability in cortisol response to kennelling has been reported in several studies [9,14]. Moreover, cortisol secretion lacks specificity as a stress response, which greatly increases the potential for misinterpretation of data [15,16]. For instance, cortisol levels have been found to increase after exercise [17,18] and excitement [19], and appear to provide an indication of arousal [16] without specifying the emotional valence of that arousal [16,20,21]. Such findings have led researchers to question the value of glucocorticoid levels as a welfare indicator e.g. [22].

Physiological indicators of stress and/or affect identified in other species might offer more reliable and specific welfare indicators in dogs than the classic stress hormones, and/or enable the valence or quality of arousal to be determined when measured alongside C/Cr. For example, the stress of immobilisation can lead to oxidative stress and damage in tissue by causing an imbalance of antioxidant status in rats [23]. Similarly, increased oxidative stress has been associated with chronic stress in humans [24], and may be implicated in the pathophysiology of depression [25]. Lipid peroxidation, of which 8-iso-prostaglandin F2a ("ISOP") [26] and thiobarbituric acid reactive substances (TBARS) [27] are products, provides a biomarker of oxidative stress [28]. Malondialdehyde (MDA) provides a further measure of lipid peroxidation [29] and has been used as a biomarker of oxidative stress in brain tissue of rabbits [27] and in plasma of dairy cows [30].

Although combining multiple physiological measures provides a means of triangulating the level and duration of an animal's stress response, husbandry staff in kennel establishments require quick, robust and economical measures of welfare. Therefore, in addition to testing nine physiological parameters in this study, we also recorded six physical and 28 behavioural measures.

Measurement of any parameter is difficult to interpret accurately without comparative baseline values and, with no single diagnostic test, an animal's welfare or quality of life should be judged on how far measurements deviate from 'normality' [31]. Nonetheless, few studies have examined the physiology and behaviour of dogs under normal home conditions [32]. To the authors' knowledge, only one published study has followed the same subjects from a home to kennel environment and only C/Cr was measured within-subjects under both conditions [5].

Therefore, the current study aimed to: (i) test the validity of a range of physiological, physical and behavioural parameters as indicators of acute, kennelling-induced, stress in dogs using a within-subjects design; (ii) establish baseline values for each parameter that reflect 'normality', as measured in dogs' normal home environment; and (iii) test for relationships between welfare indicators that are informative but difficult to conduct cheaply or quickly by husbandry staff (such as physiological parameters) and those which could easily and robustly be used by husbandry staff on a regular basis.

It was assumed that dogs would show higher levels of stress in the kennel compared to home environment, and it was predicted that this would be reflected in physiological, physical and behavioural measurements deviating from normality (baseline values) when dogs entered boarding kennels. The predicted directions of deviation are presented in Table 1.

2. Material and methods

2.1. Subjects

The subjects were 29 privately-owned dogs from 29 separate households in Northern Ireland. To test the robustness of each measurement as a general canine indicator of acute stress, we did not control for dogs' age, sex, breed or background. Subject information (i.e. age, breed, sex, known health problems, behavioural problems, history of kennelling, source [purchased as puppy from breeder; rehomed], neuter status and number of dogs in the household) was gathered from the owners.

Dogs (14 males, 15 females) were aged between 1 and 10 years (mean = 4.43 years; SD = 2.69). The neutering status of three dogs (1 male, 2 females) was unknown. Of the remainder, 65.4% (8 males, 9 females; 58.6% of total sample) were entire and 34.6% (5 males, 4 females; 31.0% of total sample) were neutered. Purebred dogs constituted 82.8% of the sample and represented 21 different breeds. Crossbreeds (offspring of purebred parents of two different breeds) and mixed-breeds (unknown parentage, or offspring of non-purebred parents) were also represented in 10.3% and 6.9% of the sample, respectively.

Two dogs had arthritis, one related to an historical injury and one related to age deterioration. Another dog had a small hole in his heart, which was not reported to have caused any health issues. The data from these three dogs were examined closely (using the 'Explore' feature of SPSS, version 19). The dogs did not represent consistent outliers in home measurement data and, so, were not excluded from the analyses. No other health problems were reported. No dogs were reported to have shown aggressive behaviour towards humans in the past, where aggressive behaviour towards humans was defined as having bitten someone on at least one occasion. Two owners reported occasional destructive behaviour in their dog when left at home alone; however, these dogs were not home alone when measurements were taken, and destructive behaviours were not observed in either environment.

Of those dogs that came from multi-dog households (41% of total sample), eight (66.7%) were kennelled with all of their home companions, two (16.7%) were kennelled with one of the two (n = 1) or three (n = 1) dogs with which they shared their home, and two (16.7%) were housed individually in the boarding kennels. To avoid selection bias in homes with more than one dog, each dog in the household was assigned a number and the subject was randomly selected using the "true random number generator" on www.random.org. In two out of 12 multi-dog households, the owners chose the focal dog because the alternative dogs showed signs of nervousness in the presence of strangers or suffered from long-term ill health.

2.1.1. Recruitment of subjects

Dog owners were recruited through future bookings at the participating boarding kennel establishments, from the staff and student population at Queen's University Belfast, and by advertisements in the monthly newsletter of one boarding kennel, a local newspaper, a pet supply store, and a veterinary clinic. All dog owners consented to all measurements being taken from their dog and no personal information about the owners was requested.

2.2. Research design

A within-subjects design was employed where measurements (see Section 2.4.1) were taken from all subjects in two different environments: (i) dogs' own homes and (ii) boarding kennels. Boarding kennels were chosen over rehoming centres to obtain true baseline (non-stressed) levels in subjects that were, presumably, already experiencing a stable