Should stress management be part of the clinical care provided to chronically ill dogs?

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Abstract

As a consequence of their physical and/or psychological effects, on-going diseases have the potential to elicit chronic stress in dogs. Chronic stress may contribute to disease progression and negatively affect welfare. By investigating whether on-going illnesses cause chronic stress in dogs and exploring the relationship between hair cortisol and potential disease-dependent and disease- independent stressors, this research aimed to determine if stress management should be integrated into veterinary care. Hair samples were collected from 33 dogs to assess cortisol levels (Ill n = 16; 12 non-black and 4 black; healthy n = 17; 12 non-black and 5 black) using a commercially available biochemical assay. In addition, a questionnaire was distributed to the owners of these dogs to gather information on pet care, chronic stress behaviours and disease characteristics. The hair cortisol levels of black and non-black dogs did not differ significantly (U = 89, df = 31, p = 0.442). Data were therefore pooled for further analysis. Significant differences were not found in the hair cortisol levels of chronically ill compared to healthy dogs (t = - 0.655, df = 30, p = 0.517) or the number of dogs with chronic stress behaviours in each group (χ^2 = 0.667, df = 1, p = 0.414). Ill dogs with disease signs or lifestyle restrictions did not have significantly different hair cortisol levels to those without them (signs: t = 0.321, df = 14, p = 0.753; lifestyle restrictions: t = 0.154, df = 14, p = 0.880). Hair cortisol was not significantly related to the number of veterinary visits (r_s = - 0.152, df = 31, p = 0.397). However, it was significantly correlated with the length of time regularly left alone in healthy and chronically ill dogs (r_s = 0.417, df = 31, p = 0.016). In addition, the hair cortisol levels of healthy dogs were significantly correlated with time regularly left alone in single dog (r_s = 0.726, df = 7, p = 0.027), but not multidog households (r_s = 0.528, df = 6, p = 0.179). Further research with a larger sample size is required to confirm our findings. Nonetheless, as chronic stress may be detrimental to the health of dogs, lifestyle factors, such as the social environment and time regularly left alone, should be taken into consideration when planning canine veterinary care.

Keywords

Dog, welfare, chronic disease, chronic stress, hair cortisol, time left alone

1. Introduction

A chronic disease is a persistent illness of more than one week's duration (Blood *et al.*, 2007). Many chronic diseases in dogs can only be managed, rather than cured, and some are also progressive (Blood *et al.*, 2007). Veterinarians often focus on physical health (Wojciechowska *et al.*, 2005) but may not consider the role of stress in the disease process and in patient welfare. This is hardly surprising, as very little research exists on the relationship between stress and disease in dogs. Previously, 2 independent research groups found that dogs with non-adrenal diseases had significantly higher acute stress levels than healthy controls (plasma cortisol levels; Church *et al.*, 1994; urinary cortisol: creatinine ratios; Kaplan *et al.*, 1995). Indeed, based on their results, Kaplan *et al.* (1995) concluded that the stress response is a necessary adaptation to disease. However, Mc Ewen (2005) notes that although short-term, moderate stress (allostasis) may be beneficial, as it allows the individual to adapt to change, prolonged or excessive stress (or allostatic overload) may contribute to disease processes. Results from a study by Dreschel (2010) appear to support this. She found that stressful behavioural conditions in dogs were predictive of skin disorders (non-social fear and separation anxiety) and a shortened lifespan (stranger-related fear) (Dreschel, 2010). Although the effects of chronic stress on canine disease processes are currently unknown, one may theorise based on the physiological effects of the stress response. The stress hormones, adrenaline and cortisol, stimulate a shift in immunity from a cellular type to a humoral type (Elenkov and Chrousos, 1999). This may increase vulnerability to infections (Korte *et al.*, 2005), trigger or exacerbate autoimmune disease (Elenkov and Chrousos, 1999) and facilitate the growth and metastasis of neoplasms (Elenkov and Chrousos, 1999). Cortisol increases blood glucose (Becker *et al.*, 2002), which may be problematic for animals with diabetes mellitus. In addition, cortisol impedes wound healing (Tennant, 2002). Prolonged sympathetic activity may have negative cardiovascular effects (arrhythmia, endothelial damage, hypertension; Esch *et al.*, 2002). Inflammatory diseases may directly stimulate stress via the release of cytokines (O'Connor *et al.*, 2000). Disease may also cause stress indirectly, through unpleasant clinical signs or undesirable lifestyle changes. Ursin and Eriksen (2004) note that stress may be caused by stimuli perceived as aversive or unmet expectations. In addition, everyday stressors unrelated to disease may affect the health of chronically ill dogs.

Previously, the confounding effects of acute stressors and the need for repeated sampling created difficulties for canine chronic stress research (Davenport *et al.*, 2006). However, hair cortisol has recently been validated as a biomarker for chronic stress in dogs (Accorsi *et al.*, 2008; Bennett and Hayssen, 2010). It is insensitive to acute stressors (Bennett and Hayssen, 2010) and provides an average of the individual's cortisol response over the period of hair growth (Accorsi *et al.*, 2008). Unless related data or precise time periods are involved, a single sample per subject is sufficient (Bennett and Hayssen, 2010). Minimal restraint is required for sampling and hair is straightforward to collect and store (Accorsi *et al.*, 2008). However, no normal range exists for dogs. In addition, as hair colour may affect cortisol content (black hair contains less cortisol than agouti hair, which contains less than yellow hair; Bennett and Hayssen, 2010), it is important to standardise coat colour. Behavioural indicators of chronic stress may be used in combination with hair cortisol, to reduce the risk of obtaining false positive or negative results (Beerda *et al.*, 1997; Dawkins, 2006). Unfortunately, the limited indicators available in the literature (Beerda *et al.*, 1999a, 2000) are based on kennelled dogs, rather than pet dogs at home. There is also significant overlap between the behavioural signs of canine chronic stress and compulsions or stereotypies. Autogrooming (Beerda *et al.*, 1999a) may also be difficult to interpret in cases with atopy. Although stereotypic behaviours may be caused by chronic stress or conflict (Luescher, 2000), they may not occur in all individuals, and once triggered, may continue in the absence of an on-going stressor (Mason and Latham, 2004). An owner-completed questionnaire can be a good method for gathering information on chronic stress behaviours in pet dogs, as it harnesses the owners' knowledge of their behaviour over time and across contexts, and avoids the observer effect (Meagher, 2009). To investigate whether stress management should be integrated into veterinary care, this research compared the chronic stress levels of chronically ill and healthy dogs and investigated potential stressors. It was hypothesized that the levels of chronically ill dogs would be significantly higher than those of healthy dogs, and would be influenced by disease-dependent and disease-independent stimuli.

2. Materials and methods

2.1 Subjects

Thirty-three dogs participated in the research, which was carried out in June 2013. All dogs were pet dogs from Dublin, Ireland. Chronically ill subjects were recruited from the charity veterinary clinic of the North County Dublin Society for the Prevention of Cruelty to Animals (NDSPCA) and healthy dogs were recruited from the NDSPCA and the general public. Both sexes were represented (14 males, 19 females) and most dogs were neutered (27 dogs, 82%). Ages ranged from 2 to 15 years and a variety of breeds were included. Chronically ill dogs (n = 16) comprised the test group and healthy dogs (n= 17) served as the control group. Of the chronically ill dogs, 5 had osteoarthritis, 5 had cardiac failure, 2 had ocular cataracts, 1 had liver disease, 1 had atopic dermatitis, 1 had chronic bronchitis and 1 had neoplasia (perianal adenoma or carcinoma; not diagnosed histopathologically) and osteoarthritis. As Bennett and Hayssen (2010) found that black hairs contained significantly less cortisol than yellow (non-black) hairs, dogs were also initially subcategorised based on hair colour. Therefore, 4 groups were created; chronically ill non-black-haired dogs (n= 12; 9 females, 3 males), chronically ill black-haired dogs (n= 4; 3 females, 1 male), healthy non-black-haired dogs (n= 12; 4 females, 8 males) and healthy black-haired dogs (n= 5; 3 females, 2 males).

2.2 Inclusion criteria

For dogs to be included in the study a number of criteria needed to be met. Chronically ill and healthy dogs were required to be older than two years of age, as cortisol levels are significantly lower in puppies compared to adult or geriatric dogs (Palazzolo and Quadri, 1987). To ensure that each owner was fully aware of their dog's health status, dogs must have been in their owners' possession for at least three months prior to the commencement of the study. Dogs with agouti hairs could not participate, as agouti hairs contain a moderate amount of cortisol (Bennett and Hayssen, 2010) and may confound the interpretation of statistical test results. Dogs who had suffered from acute illnesses within the previous three months were excluded from participation, as acute illnesses may affect cortisol levels (Church *et al.*, 1994; Kaplan *et al.*, 1995). Recent experience of acute illness was determined by history taking. Dogs in the healthy group must have had a non-remarkable veterinary examination within the previous year. Additional inclusion criteria also applied to chronically ill dogs. To participate, a veterinarian must have diagnosed their chronic illness 3 or more months before the start of the study. As hair grows at the rate of approximately one centimetre per month (Wennig, 2000), the latter precaution was included to avoid accidentally sampling hair growth from the period before the onset of disease. Dogs with hormonal disease (apart from diabetes mellitus), or those on medications that could affect cortisol levels (for example, steroids, phenobarbitone, progestagens) or interfere with the assay were also excluded from participation.

2.3 Behaviour, Health and Lifestyle Questionnaire

The owner of each dog was asked to complete a behaviour and health questionnaire. The content validity of the questionnaire had previously been confirmed by a behaviourist and by an experienced veterinarian; and its test-retest reliability was also found to be excellent (r_s= 0.97, df = 5, p<0.001). Owners were asked to score their dog on a presence or absence scale of behavioural indicators of canine chronic stress (Table 1) and to indicate when the behaviour was first observed. These indicators were obtained from Beerda *et al.* (1999a) and Luescher (2004). However, 4 chronic stress behaviours (vocalisation, changes in locomotion, a low posture and paw lifting; Beerda *et al.*, 1999a) were excluded from the questionnaire due to their lack of specificity to chronic stress (Beerda *et al.*, 2000). Owners of chronically ill dogs were asked to provide information on disease type, duration of illness and medications being administered. Disease signs were scored on a presence or absence scale and lifestyle restrictions caused by disease were scored on an agree or disagree scale (Table 1). In addition, owners of all dogs were asked to indicate the number of trips to the vet or periods of hospitalisation the dogs had experienced during the previous year.

Table 1. Behaviour, health and lifestyle questions.

Behaviours (Does your dog carry out any of the following behaviours regularly?)	**Signs/symptoms (Does your dog regularly display any of the following symptoms?)**	**Lifestyle restrictions (Since becoming ill, my dog…)**
Licking paws causing redness and/or irritation or injury	Pain	Doesn't want to play as much as before
Grooming (lick, bite, scratch or suck) other areas of the body causing redness and/or irritation or injury.	Vomiting	His/her ability to exercise has reduced
Tail chasing (if behaviour not trained by owner)	Diarrhoea	Is not as eager to eat as before
Eating own faeces	Breathing problems	Growls, snaps or bites at dogs more than before
Eating the faeces of another dog	Coughing	Growls, snaps or bites at people more than before
Snapping at the air (not at a toy, person or animal)	Passing urine often	Sits next to family members less than before
Chasing light beams (when not playing with owner)	Drinking a lot	
Turning repeatedly in a circle on the one spot (when not playing or trying to lie down)	Difficulty getting comfortable when sitting or lying down	
Chasing shadows		
Suddenly snapping or biting at itself		
Suddenly turning and staring intently at its rear end		

Behavioural indicators of chronic stress were based on those observed by Beerda *et al.* (1999a) and Luescher (2004). Items were scored on a presence/absence (behaviours and signs) or agree/disagree (lifestyle) scale.

2.4 Hair sampling

Hair samples were taken from all dogs by brushing (short-haired dogs) or trimming (long-haired dogs); approximately 300mg was obtained per subject. The cortisol extraction technique was adapted from that of Bennett and Hayssen (2010) and Davenport *et al.* (2006). Hair samples were not washed before the extraction process as washing may remove cortisol from the interior of the hair shaft (Davenport *et al.*, 2006, Gow *et al.*, 2010). The hair was powdered to maximise cortisol recovery (Davenport *et al.*, 2006). Hair cortisol concentrations were determined using a DRG Diagnostics salivary cortisol ELISA test kit (DRG Instruments GMBH, Marburg, Germany) with a sensitivity of 0.537ng/ml (DRG Diagnostics, 2007). Units (ng/ml) were subsequently converted to pg/mg hair.

2.5 Statistical analyses

All statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 21 (IBM Corporation, Armonk, NY). The level of significance was set at $p < 0.05$. The Chi Square test was used for categorical data (comparison of the number of stress behaviours performed by dogs in the chronically ill or healthy groups). Parametric tests were used for ratio or interval data when the conditions for normality (determined by Kolmogorov-Smirnov test) and equality of variance (determined by Levene's test) were met. Parametric tests were therefore performed to test for a significant difference in the ages of ill and healthy dogs and the hair cortisol levels of ill and healthy dogs, dog with and without chronic stress behaviours, ill dogs with and without current signs or lifestyle restrictions, male and female dogs and dogs from single or multidog households (Independent samples t test). A parametric test was also selected to search for a significant relationship between age and hair cortisol (Pearson correlation) and between time regularly left alone and the presence or absence of chronic stress behaviours (logistic regression). When the conditions for normality were not met, non-parametric tests were selected (Mann-Whitney U test for the hair cortisol levels of black-haired and non-black-haired dogs and the number of trips made to the vet by chronically ill and healthy dogs; Spearman rank correlation for the relationship between time regularly left alone and hair cortisol levels, including in single and multidog households).

3. Results

3.1 Hair colour and cortisol levels

No significant difference was found in the hair cortisol levels of black and non-black dogs ($U = 89$, $df = 31$, $p = 0.442$). Therefore, hair colour data were pooled for further analysis.

3.2 Chronic stress levels of chronically ill and healthy dogs

Subject characteristics and individual hair cortisol levels are displayed in Tables 2 (ill dogs) and 3 (healthy dogs). The hair cortisol concentrations of the ill dogs ranged from 1.77pg/mg to 42.82pg/mg (15.22pg/mg ± 10.52; mean ± standard deviation). Excluding an outlier (42.82pg/mg) the range was 1.77pg/mg to 25.22pg/mg, with a mean and standard deviation of 13.38 pg /mg ± 7.7. The hair cortisol of the healthy dogs ranged from 1.70pg/mg to 28.79pg/mg (17.48pg/mg ± 8.95). The hair cortisol levels of the two groups did not differ significantly ($t = -0.655$, $df = 30$, $p = 0.517$) (the outlier was included in the statistical analysis). Overall, only 18% of dogs displayed chronic stress behaviours; this included 12.5% of chronically ill dogs (2 dogs) and 23.5% of healthy dogs (4 dogs) (see Tables 2 and 3). No significant difference was found in the number of chronic stress behaviours displayed in each group ($\chi^2 = 0.667$, $df = 1$, $p = 0.414$). In addition, dogs performing chronic stress behaviours did not have significantly different hair cortisol concentrations to those not performing them ($t = 1.377$, $df = 31$, $p = 0.179$).

3.3 Disease-dependent factors and hair cortisol levels

Of the chronically ill dogs, 62.5% (10 dogs) experienced signs of clinical disease (see Table 2). However, dogs with clinical signs did not have significantly different hair cortisol levels to those without them ($t = 0.321$, $df = 14$, $p = 0.753$). Eighty-one per cent of chronically ill dogs (13) had lifestyle restrictions imposed by disease. Dogs experiencing lifestyle restrictions did not have significantly different hair cortisol concentrations to dogs not experiencing these ($t = 0.154$, $df = 14$, $p = 0.880$). Three ill dogs but no healthy dogs had been hospitalised within the previous year. Unsurprisingly, chronically ill dogs visited the vet highly significantly more often than healthy dogs ($U = 52$, $df = 31$, $p = 0.001$). However, hair cortisol levels were not significantly related to the frequency of visitation ($r_s = -0.152$, $df = 31$, $p = 0.397$).

Table 2: Characteristics and hair cortisol levels of ill dogs

Subject No.	Breed	Disease type	Age (yrs.)	Sex	Neuter (Y/N)	Hair cortisol (pg/mg)	No. chronic Stress behaviours	No. of symptoms/ signs	Type of symptoms/ signs	No. of lifestyle restrictions
1	Crossbreed	Cardiac failure	9	F	Y	1.77	0	1	Dyspnoea	2
2	Crossbreed	Osteoarthritis	9	F	Y	20.80	0	2	Pain	1
3	Labrador Retriever	Osteoarthritis	13	F	Y	7.92	0	1	Pain	1
4	Labrador Retriever	Chronic Bronchitis	10	F	Y	11.05	0	1	Coughing	1
5	Springer Spaniel	Cardiac failure	14	F	Y	10.87	0	2	Polydipsia Discomfort	2
6	Crossbreed	Osteoarthritis	7	F	Y	10.72	0	0	None	1
7	Crossbreed	Atopy	4	F	Y	3.03	3	0	None	1
8	Shih Tzu	Cardiac failure	9	M	N	7.66	1	3	Diarrhoea Coughing Polyuria	1
9	Crossbreed	Neoplasia and osteoarthritis	15	M	Y	42.82	0	3	Coughing Tachypnoea Discomfort	1
10	Crossbreed	Ocular cataracts	2	F	Y	25.22	0	0	None	0
11	King Charles Spaniel	Cardiac failure	10	M	N	3.31	0	5	Diarrhoea Dyspnoea Coughing Polyuria Polydipsia	0
12	Crossbreed	Liver disease	10	F	Y	19.22	0	2	Polyuria Polydipsia	4
14	Jack Russell Terrier	Cardiac failure	13	F	Y	19.75	0	0	None	0
15	Labrador Retriever	Ocular cataracts	10	F	Y	23.56	0	0	None	1
16	Crossbreed	Osteoarthritis	6	F	Y	15.79	0	0	None	1
17	Crossbreed	Osteoarthritis	8	M	Y	20.02	0	1	Pain	2

Table 3: Characteristics and hair cortisol levels of healthy dogs

Subject No.	Breed	Age (yrs.)	Sex	Neuter (Y/N)	Hair Cortisol (pg/mg)	No. Chronic Stress behaviours
19	Labrador Retriever	6	F	Y	17.48	0
20	Border Collie	7	M	Y	25.56	0
21	King Charles Spaniel	6	F	Y	20.23	0
22	Terrier (West Highland)	5	M	Y	17.66	0
23	Cocker Spaniel	5	M	N	12.58	0
24	Springer Spaniel	4	M	Y	28.50	0
25	Bichon Frise	2	F	N	4.33	0
26	Akita	5	F	N	28.79	0
27	Crossbreed	2	M	N	27.73	1
28	Labrador Retriever	5	M	Y	16.67	1
29	Crossbreed	2	M	Y	9.55	1
30	Crossbreed	5	M	Y	1.70	0
33	Labrador Retriever	10	M	Y	23.64	0
36	Springer Spaniel	2	F	Y	18.14	0
38	Crossbreed	6	F	Y	3.67	2
39	Golden Retriever	2	M	Y	23.48	0
40	Border Collie	7	F	N	10.87	0

M, male; N, no; F, female; Y, yes

3.4 Disease-independent factors and hair cortisol levels

There was no significant difference in the hair cortisol levels of male and female dogs ($t = 1.274$, $df = 31$, $p = 0.212$). Ill dogs were highly significantly older than healthy dogs (mean age of ill dogs = 9.31 years +/- 3.497 standard deviation; mean age of healthy dogs = 4.63 years +/- 2.247 standard deviation; $t = 4.470$, $df = 31$, $p < 0.001$). However, hair cortisol levels were not significantly related to age ($r = 0.666$, $df = 31$, $p = 0.714$). Chronically ill dogs were left alone for a mean time of 3.5 hours (± 3.16 standard deviation) and the entire sample group was left alone for a mean time of 3.7 hours (± 2.87 standard deviation). There was no significant correlation between the hair cortisol concentrations of chronically ill dogs and the length of time they were regularly left alone ($r_s = 0.276$, $df = 14$, $p = 0.30$). However, when the subject groups were considered together (both healthy and chronically ill subjects), hair cortisol levels were significantly and positively related to the length of time regularly left alone ($r_s = 0.417$, $df = 31$, $p = 0.016$). In contrast, time regularly left alone could not significantly predict the presence of chronic stress behaviours (odds ratio = 0.862, $p = 0.416$). No significant difference was found in the hair cortisol levels of dogs from single or multiple dog households ($t = -0.803$, $df = 31$, $p = 0.428$) and hair cortisol levels were not significantly correlated with time regularly left alone in either single ($r_s = 0.452$, $df = 12$, $p = 0.104$) or multidog households ($r_s = 0.400$, $df = 17$, $p = 0.089$). However, the hair cortisol levels of healthy dogs were significantly correlated with time regularly left alone in single ($r_s = 0.726$, $df = 7$, $p = 0.027$), but not multidog households ($r_s = 0.528$, $df = 6$, $p = 0.179$).

4. Discussion

4.1 Hair colour and cortisol levels

In this study, hair cortisol concentrations did not significantly differ between black-haired and non-black-haired subjects. This contrasts with the results of Bennett and Hayssen (2010), who found that the hair cortisol levels of black dogs were significantly lower than those of non-black dogs. However, Bennett and Hayssen (2010) only studied German shepherd dogs and Labrador retrievers, while our subject group contained a greater variety of breeds, including crossbreeds. Our finding may simplify the experimental design of future research, as dogs with black hair may not need to be considered separately to those with non-black hair.

4.2 Chronic stress state of subjects

This research found that the hair cortisol levels of healthy and chronically ill dogs did not differ significantly. Chronic stress behaviours were only performed by a small number of dogs and there was no significant difference in their prevalence in each group. These results suggest that either both groups were chronically stressed or that neither were chronically stressed. The lack of a normal range of canine hair cortisol, the current scarcity of published research applying this technique, and the lack of standardisation in hair cortisol extraction and assay methods, create difficulties for interpretation. However imperfect, comparison to other studies is presently the only aid to interpretation. Bennett and Hayssen (2010) used similar cortisol extraction and assay techniques as the present study and had results similar to our findings [Bennett and Hayssen, 2010; old hair growth; 12.63 ± 5.45pg/mg mean ± standard deviation; present study; 15.22pg/mg ± 10.52; mean ± standard deviation (ill), 17.48pg/mg ± 8.95 (healthy)]. Although the health status of their subject dogs was not disclosed, their sample group were living in a home environment and the majority were not exposed to any major stressors (with the potential exception of 2 dogs who had recently weaned puppies and 2 dogs who were guide dogs). Accorsi *et al.* (2008) found a much lower mean hair cortisol concentration in dogs (2.10 ± 0.22pg/mg). However, their hair samples were minced, rather than powdered, and this may result in lower cortisol extraction yields (Bennett and Hayssen, 2010). In addition, they used a radioimmunoassay rather than an enzyme immunoassay and this too may account for the disparity in results (Bennett and Hayssen, 2010). Although Siniscalchi *et al.* (2013) used the same cortisol extraction and assay techniques as Accorsi *et al.* (2008) they found much higher hair cortisol levels (10:00 hour = 65.53pg/mg + 21.49 mean + standard error; 17:00 hour = 96.01pg/mg + 9.57; originally presented in pM/g). Indeed their results were also much higher than those of Bennett and Hayssen (2010), and those of the current study. However, Siniscalchi *et al.* (2013) exposed their subjects to various acoustic stimuli, including noise from a simulated thunderstorm, and hair cortisol was measured two weeks later. At 9 am hair cortisol levels were significantly and positively correlated with acute stress behaviours displayed during stimuli presentation, likely reflecting a state of chronic stress caused by the sounds (Siniscalchi *et al.*, 2013). As the hair cortisol levels from dogs in a normal home environment (Bennett and Hayssen, 2010) were similar to those in our study and as the levels of stressed dogs (Siniscalchi *et al.*, 2013) were much higher, we may deduce that the subjects in our research were not chronically stressed. The absence of a chronic stress response to on-going disease is curious given that Church *et al.* (1994) and Kaplan *et al.* (1995) found significantly higher cortisol levels in ill dogs compared to healthy dogs. However, the cortisol sampling methods employed by these studies (plasma cortisol, Church *et al.*, 1994; urinary cortisol/creatinine ratio, Kaplan *et al.*, 1995) identify acute stress but not chronic stress (Davenport *et al.*, 2006). Also, neither research group distinguished acutely ill from chronically ill dogs for the purposes of testing (Church *et al.*, 1994; Kaplan *et al.*, 1995). One of our additional findings was that dogs performing chronic stress behaviours did not have significantly higher hair cortisol levels than dogs not performing any. The concept of animal "coping styles" (Koolhaas *et al.*, 1999) may provide an explanation for this. Animals with proactive coping styles mount a sympathetic response to stress and are likely to react actively to stressors (Koolhaas *et al.*, 1999). This may predispose them to the development of stereotypies (Koolhaas *et al.*, 1999). In contrast, animals with reactive coping styles are likely to mount a cortisol response and avoid or withdraw from a stressor (Koolhaas *et al*., 1999).

4.3 Disease-dependent factors and chronic stress

A variety of diseases were included in our study (see Table 2). These included both progressive and nonprogressive diseases, of inflammatory and noninflammatory origin. Inflammatory diseases may directly trigger a stress response via the release of mediators such as cytokines (O'Connor *et al.*, 2000). However, the consequences of any disease type may stimulate a stress response if negatively perceived by the animal (Ursin and Eriksen, 2004). In humans, it has been observed that individuals with chronic pain have significantly higher hair cortisol levels and perceived stress scores than pain-free controls (Van Uum *et al.*, 2008). In addition, nausea was significantly correlated with perceived stress levels in pregnant women (Chou *et al.*, 2008). However, in our research, ill dogs with clinical signs did not have significantly different hair cortisol levels to those without clinical signs. It is possible that some clinical signs (such as pain, vomiting or dyspnoea) may be inherently more stressful than others (such as polyuria and polydipsia), or that clinical signs are acutely or intermittently stressful. Alternatively, our small sample size may have influenced our results. In addition, some signs reported as being disease related may actually be side effects attributable to the patient's medication (for example; polyuria and polydipsia may be caused by diuretics administered in cardiac failure). However, these too could cause stress if negatively perceived. As a discrepancy between an animal's environment and its expectations may stimulate stress (Dantzer and Mormède, 1983; Ursin and Eriksen, 2004), one might also expect on-going lifestyle restrictions to be a source of chronic stress. However, the hair cortisol concentrations of dogs with lifestyle restrictions were not significantly higher than those without them. Once again, however, our sample size may have affected our results. Disease severity (including severity of clinical signs and lifestyle restrictions) may be an important factor to consider, as Kaplan *et al.* (1995) observed that dogs with severe disease had significantly higher serum cortisol levels than dogs with mild to moderate disease. Our research did not specifically investigate this, because of the difficulties involved in gathering data for this purpose in multiple disease types. However, we did notice that one subject had a particularly high hair cortisol level (42.82 pg/mg). This subject was diagnosed with osteoarthritis and locally invasive anal neoplasia (possibly adenocarcinoma), on the basis of physical examination. His high hair cortisol concentration could be explained by the severity of his diseases and/or the presence of multiple disease types. Future research could investigate the relationship between disease severity and canine chronic stress and this may be simplified by focusing on single disease states. As none of our healthy subjects had been hospitalised within the previous year we were unable to study its effect on hair cortisol concentrations. However, we did examine the effect of visiting the veterinary clinic. Given that 78.5% of dogs display fearful behaviour upon visiting the veterinary clinic (Döring *et al.*, 2009), it was surprising that in our subjects, hair cortisol was not significantly related to the number of trips made to the veterinarian during the previous year. This may be explained by habituation or by the triggering of only an acute stress response. Alternatively, it is likely that the samples collected were not representative of a full year's hair growth.

4.4 Disease-independent factors and chronic stress

In our research we also investigated the effects of disease-independent variables on chronic stress levels. These included; age, gender, time left alone and the effect of living in a single or multidog household. Although our chronically ill subjects were highly significantly older than our healthy subjects, hair cortisol was not significantly related to age. This is in agreement with Palazzolo and Quadri (1987), who found no significant difference in the mean plasma cortisol levels of adult and old dogs. Although gender is not itself a stressor, it may influence stressor perception. Beerda *et al.* (1999b) observed that bitches had greater stress sensitivity than dogs, as demonstrated by an increased salivary cortisol response to a sound blast and higher cortisol induction by corticotrophin releasing hormone. Therefore, gender could ameliorate or enhance the effect of a disease-related or independent stressor. However, this hypothesis could not be confirmed by the current research, as there was no significant difference in the hair cortisol levels of male and female dogs. Rehn and Keeling (2011) observed that canine greeting behaviour intensified with an increasing length of owner absence, but were unable to attribute this to distress caused by separation. Within our research, the hair cortisol levels of chronically ill dogs were not significantly related to time regularly left alone. However, this was a small sample group. When healthy dogs were considered in addition to chronically ill dogs, hair cortisol levels were significantly and positively correlated with the length of time regularly left alone. However, time regularly left alone could not significantly predict the presence of chronic stress behaviours. To our best knowledge, this is the first indicator that canine chronic stress levels may be affected by the duration of owner absence. Because time regularly left alone accounts for only 17% of the variability in hair cortisol, this should be interpreted with caution and future research may be required to confirm this finding. Previous research found significantly higher stress levels (Bennett and Hayssen, 2010; hair cortisol levels), or a tendency towards this (Dreschel and Granger, 2005; salivary cortisol levels), in multidog households compared to single dog households. In contrast, we did not find a significant difference in the hair cortisol levels of dogs from single or multidog households. Moreover, we identified a significant and positive interaction between time regularly left alone and the hair cortisol levels of healthy dogs in single dog but not multidog households. Canine relationships can be complex and it is possible that there was low social stress within our multidog households. The company of other dogs may also act as a buffer against the stress of being left alone. In the study of Dreschel and Granger (2005), the salivary cortisol levels of dogs from multidog households increased significantly less than those from single dog households after exposure to a recorded thunderstorm. However, owing to our small sample size and the lack of a similar finding in our pooled subject groups, it is difficult to interpret the significance of this result. Nonetheless, it would seem sensible to minimise time regularly left alone, especially if the dog is living in a single dog household, as chronic stress may be detrimental to health (Dreschel, 2010; McEwen, 2005).

5. Conclusions

Hair cortisol analysis has the potential to be an excellent biomarker of canine chronic stress, as it is unaffected by acute stressors and can determine individual chronic stress levels from single samples. The results from our study suggest that on-going diseases do not cause chronic stress in dogs. However, additional research is required to confirm our findings in individual disease states and to investigate the effect of disease severity on canine chronic stress levels. Time regularly left alone may affect the chronic stress levels of both healthy and chronically ill dogs and living in a single dog household may interact with time regularly left alone to influence the chronic stress levels of healthy dogs. However, further research with a larger sample size is needed to support our results. Nonetheless, as chronic stress may be detrimental to the health of dogs, lifestyle factors, such as the social environment and time regularly left alone, should be taken into consideration when planning canine clinical care.

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Ethical considerations

The Ethics and Welfare committee of Bishop Burton College approved this study in advance.

Conflict of Interest statement

The authors declare that there is no known conflict of interest associated with this research and there has been no significant financial support for this work that could have influenced its outcome.

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