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

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1 **Abstract**

2 As a consequence of their physical and/or psychological effects, on-going diseases
3 have the potential to elicit chronic stress in dogs. Chronic stress may contribute to
4 disease progression and negatively affect welfare. By investigating whether on-
5 going illnesses cause chronic stress in dogs and exploring the relationship between
6 hair cortisol and potential disease-dependent and disease-independent stressors,
7 this research aimed to determine if stress management should be integrated into
8 veterinary care. Hair samples were collected from 33 dogs to assess cortisol levels
9 (Ill n = 16; 12 non-black and 4 black; healthy n = 17; 12 non-black and 5 black)
10 using a commercially available biochemical assay. In addition, a questionnaire was
11 distributed to the owners of these dogs to gather information on pet care, chronic
12 stress behaviours and disease characteristics. The hair cortisol levels of black and
13 non-black dogs did not differ significantly ($U = 89$, $df = 31$, $p = 0.442$). Data were
14 therefore pooled for further analysis. Significant differences were not found in the
15 hair cortisol levels of chronically ill compared to healthy dogs ($t = -0.655$, $df = 30$, p
16 $= 0.517$) or the number of dogs with chronic stress behaviours in each group ($\chi^2 =$
17 0.667 , $df = 1$, $p = 0.414$). Ill dogs with disease signs or lifestyle restrictions did not
18 have significantly different hair cortisol levels to those without them (signs: $t = 0.321$,
19 $df = 14$, $p = 0.753$; lifestyle restrictions: $t = 0.154$, $df = 14$, $p = 0.880$). Hair cortisol
20 was not significantly related to the number of veterinary visits ($r_s = -0.152$, $df = 31$, p
21 $= 0.397$). However, it was significantly correlated with the length of time regularly left
22 alone in healthy and chronically ill dogs ($r_s = 0.417$, $df = 31$, $p = 0.016$). In addition,
23 the hair cortisol levels of healthy dogs were significantly correlated with time
24 regularly left alone in single dog ($r_s = 0.726$, $df = 7$, $p = 0.027$), but not multidog
25 households ($r_s = 0.528$, $df = 6$, $p = 0.179$). Further research with a larger sample
26 size is required to confirm our findings. Nonetheless, as chronic stress may be
27 detrimental to the health of dogs, lifestyle factors, such as the social environment
28 and time regularly left alone, should be taken into consideration when planning
29 canine veterinary care.

30

31

32 **Keywords**

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

34

35 **1. Introduction**

36

37 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,
38 *al.*, 2007). Many chronic diseases in dogs can only be managed, rather than cured,
39 and some are also progressive (Blood *et al.*, 2007). Veterinarians often focus on
40 physical health (Wojciechowska *et al.*, 2005) but may not consider the role of stress
41 in the disease process and in patient welfare. This is hardly surprising, as very little
42 research exists on the relationship between stress and disease in dogs. Previously,
43 2 independent research groups found that dogs with non-adrenal diseases had
44 significantly higher acute stress levels than healthy controls (plasma cortisol levels;
45 Church *et al.*, 1994; urinary cortisol: creatinine ratios; Kaplan *et al.*, 1995). Indeed,
46 based on their results, Kaplan *et al.* (1995) concluded that the stress response is a
47 necessary adaptation to disease. However, Mc Ewen (2005) notes that although
48 short-term, moderate stress (allostasis) may be beneficial, as it allows the individual
49 to adapt to change, prolonged or excessive stress (or allostatic overload) may
50 contribute to disease processes. Results from a study by Dreschel (2010) appear to
51 support this. She found that stressful behavioural conditions in dogs were predictive
52 of skin disorders (non-social fear and separation anxiety) and a shortened lifespan
53 (stranger-related fear) (Dreschel, 2010). Although the effects of chronic stress on
54 canine disease processes are currently unknown, one may theorise based on the
55 physiological effects of the stress response. The stress hormones, adrenaline and
56 cortisol, stimulate a shift in immunity from a cellular type to a humoral type (Elenkov
57 and Chrousos, 1999). This may increase vulnerability to infections (Korte *et al.*,
58 2005), trigger or exacerbate autoimmune disease (Elenkov and Chrousos, 1999)
59 and facilitate the growth and metastasis of neoplasms (Elenkov and Chrousos,
60 1999). Cortisol increases blood glucose (Becker *et al.*, 2002), which may be
61 problematic for animals with diabetes mellitus. In addition, cortisol impedes wound
62 healing (Tennant, 2002). Prolonged sympathetic activity may have negative
63 cardiovascular effects (arrhythmia, endothelial damage, hypertension; Esch *et al.*,
64 2002). Inflammatory diseases may directly stimulate stress via the release of
65 cytokines (O'Connor *et al.*, 2000). Disease may also cause stress indirectly, through
66 unpleasant clinical signs or undesirable lifestyle changes. Ursin and Eriksen (2004)
67 note that stress may be caused by stimuli perceived as aversive or unmet
68 expectations. In addition, everyday stressors unrelated to disease may affect the
69 health of chronically ill dogs.

70 Previously, the confounding effects of acute stressors and the need for repeated
71 sampling created difficulties for canine chronic stress research (Davenport *et al.*,
72 2006). However, hair cortisol has recently been validated as a biomarker for chronic
73 stress in dogs (Accorsi *et al.*, 2008; Bennett and Hayssen, 2010). It is insensitive to
74 acute stressors (Bennett and Hayssen, 2010) and provides an average of the
75 individual's cortisol response over the period of hair growth (Accorsi *et al.*, 2008).
76 Unless related data or precise time periods are involved, a single sample per
77 subject is sufficient (Bennett and Hayssen, 2010). Minimal restraint is required for
78 sampling and hair is straightforward to collect and store (Accorsi *et al.*, 2008).
79 However, no normal range exists for dogs. In addition, as hair colour may affect
80 cortisol content (black hair contains less cortisol than agouti hair, which contains
81 less than yellow hair; Bennett and Hayssen, 2010), it is important to standardise
82 coat colour. Behavioural indicators of chronic stress may be used in combination
83 with hair cortisol, to reduce the risk of obtaining false positive or negative results
84 (Beerda *et al.*, 1997; Dawkins, 2006). Unfortunately, the limited indicators available
85 in the literature (Beerda *et al.*, 1999a, 2000) are based on kennelled dogs, rather
86 than pet dogs at home. There is also significant overlap between the behavioural
87 signs of canine chronic stress and compulsions or stereotypies. Autogrooming
88 (Beerda *et al.*, 1999a) may also be difficult to interpret in cases with atopy. Although
89 stereotypic behaviours may be caused by chronic stress or conflict (Luescher,
90 2000), they may not occur in all individuals, and once triggered, may continue in the
91 absence of an on-going stressor (Mason and Latham, 2004). An owner-completed
92 questionnaire can be a good method for gathering information on chronic stress
93 behaviours in pet dogs, as it harnesses the owners' knowledge of their behaviour
94 over time and across contexts, and avoids the observer effect (Meagher, 2009). To
95 investigate whether stress management should be integrated into veterinary care,
96 this research compared the chronic stress levels of chronically ill and healthy dogs
97 and investigated potential stressors. It was hypothesized that the levels of
98 chronically ill dogs would be significantly higher than those of healthy dogs, and
99 would be influenced by disease-dependent and disease-independent stimuli.

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106 **2. Materials and methods**

107 **2.1 Subjects**

108 Thirty-three dogs participated in the research, which was carried out in June 2013.
109 All dogs were pet dogs from Dublin, Ireland. Chronically ill subjects were recruited
110 from the charity veterinary clinic of the North County Dublin Society for the
111 Prevention of Cruelty to Animals (NDSPCA) and healthy dogs were recruited from
112 the NDSPCA and the general public. Both sexes were represented (14 males, 19
113 females) and most dogs were neutered (27 dogs, 82%). Ages ranged from 2 to 15
114 years and a variety of breeds were included. Chronically ill dogs (n = 16) comprised
115 the test group and healthy dogs (n= 17) served as the control group. Of the
116 chronically ill dogs, 5 had osteoarthritis, 5 had cardiac failure, 2 had ocular
117 cataracts, 1 had liver disease, 1 had atopic dermatitis, 1 had chronic bronchitis and
118 1 had neoplasia (perianal adenoma or carcinoma; not diagnosed
119 histopathologically) and osteoarthritis. As Bennett and Hayssen (2010) found that
120 black hairs contained significantly less cortisol than yellow (non-black) hairs, dogs
121 were also initially subcategorised based on hair colour. Therefore, 4 groups were
122 created; chronically ill non-black-haired dogs (n= 12; 9 females, 3 males),
123 chronically ill black-haired dogs (n= 4; 3 females, 1 male), healthy non-black-haired
124 dogs (n= 12; 4 females, 8 males) and healthy black-haired dogs (n= 5; 3 females, 2
125 males).

126

127 **2.2 Inclusion criteria**

128 For dogs to be included in the study a number of criteria needed to be met.
129 Chronically ill and healthy dogs were required to be older than two years of age, as
130 cortisol levels are significantly lower in puppies compared to adult or geriatric dogs
131 (Palazzolo and Quadri, 1987). To ensure that each owner was fully aware of their
132 dog's health status, dogs must have been in their owners' possession for at least
133 three months prior to the commencement of the study. Dogs with agouti hairs could
134 not participate, as agouti hairs contain a moderate amount of cortisol (Bennett and
135 Hayssen, 2010) and may confound the interpretation of statistical test results. Dogs
136 who had suffered from acute illnesses within the previous three months were
137 excluded from participation, as acute illnesses may affect cortisol levels (Church *et*
138 *al.*, 1994; Kaplan *et al.*, 1995). Recent experience of acute illness was determined
139 by history taking. Dogs in the healthy group must have had a non-remarkable
140 veterinary examination within the previous year. Additional inclusion criteria also

141 applied to chronically ill dogs. To participate, a veterinarian must have diagnosed
142 their chronic illness 3 or more months before the start of the study. As hair grows at
143 the rate of approximately one centimetre per month (Wennig, 2000), the latter
144 precaution was included to avoid accidentally sampling hair growth from the period
145 before the onset of disease. Dogs with hormonal disease (apart from diabetes
146 mellitus), or those on medications that could affect cortisol levels (for example,
147 steroids, phenobarbitone, progestagens) or interfere with the assay were also
148 excluded from participation.
149

150 **2.3 Behaviour, Health and Lifestyle Questionnaire**

151 The owner of each dog was asked to complete a behaviour and health
152 questionnaire. The content validity of the questionnaire had previously been
153 confirmed by a behaviourist and by an experienced veterinarian; and its test-retest
154 reliability was also found to be excellent ($r_s = 0.97$, $df = 5$, $p < 0.001$). Owners were
155 asked to score their dog on a presence or absence scale of behavioural indicators
156 of canine chronic stress (Table 1) and to indicate when the behaviour was first
157 observed. These indicators were obtained from Beerda *et al.* (1999a) and Luescher
158 (2004). However, 4 chronic stress behaviours (vocalisation, changes in locomotion,
159 a low posture and paw lifting; Beerda *et al.*, 1999a) were excluded from the
160 questionnaire due to their lack of specificity to chronic stress (Beerda *et al.*, 2000).
161 Owners of chronically ill dogs were asked to provide information on disease type,
162 duration of illness and medications being administered. Disease signs were scored
163 on a presence or absence scale and lifestyle restrictions caused by disease were
164 scored on an agree or disagree scale (Table 1). In addition, owners of all dogs were
165 asked to indicate the number of trips to the vet or periods of hospitalisation the dogs
166 had experienced during the previous year.

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

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

170

171 **2.4 Hair sampling**

172 Hair samples were taken from all dogs by brushing (short-haired dogs) or trimming
173 (long-haired dogs); approximately 300mg was obtained per subject. The cortisol
174 extraction technique was adapted from that of Bennett and Hayssen (2010) and
175 Davenport *et al.* (2006). Hair samples were not washed before the extraction
176 process as washing may remove cortisol from the interior of the hair shaft
177 (Davenport *et al.*, 2006, Gow *et al.*, 2010). The hair was powdered to maximise
178 cortisol recovery (Davenport *et al.*, 2006). Hair cortisol concentrations were
179 determined using a DRG Diagnostics salivary cortisol ELISA test kit (DRG
180 Instruments GMBH, Marburg, Germany) with a sensitivity of 0.537ng/ml (DRG
181 Diagnostics, 2007). Units (ng/ml) were subsequently converted to pg/mg hair.
182

183 **2.5 Statistical analyses**

184 All statistical analysis was carried out using IBM SPSS Statistics for Windows,
185 Version 21 (IBM Corporation, Armonk, NY). The level of significance was set at $p <$
186 0.05. The Chi Square test was used for categorical data (comparison of the number
187 of stress behaviours performed by dogs in the chronically ill or healthy groups).
188 Parametric tests were used for ratio or interval data when the conditions for
189 normality (determined by Kolmogorov-Smirnov test) and equality of variance
190 (determined by Levene's test) were met. Parametric tests were therefore performed
191 to test for a significant difference in the ages of ill and healthy dogs and the hair
192 cortisol levels of ill and healthy dogs, dog with and without chronic stress
193 behaviours, ill dogs with and without current signs or lifestyle restrictions, male and
194 female dogs and dogs from single or multidog households (Independent samples t
195 test). A parametric test was also selected to search for a significant relationship
196 between age and hair cortisol (Pearson correlation) and between time regularly left
197 alone and the presence or absence of chronic stress behaviours (logistic
198 regression). When the conditions for normality were not met, non-parametric tests
199 were selected (Mann-Whitney U test for the hair cortisol levels of black-haired and
200 non-black-haired dogs and the number of trips made to the vet by chronically ill and
201 healthy dogs; Spearman rank correlation for the relationship between time regularly
202 left alone and hair cortisol levels, including in single and multidog households).
203

204 **3. Results**

205 **3.1 Hair colour and cortisol levels**

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

209

210 **3.2 Chronic stress levels of chronically ill and healthy dogs**

211 Subject characteristics and individual hair cortisol levels are displayed in Tables 2 (ill
212 dogs) and 3 (healthy dogs). The hair cortisol concentrations of the ill dogs ranged
213 from 1.77pg/mg to 42.82pg/mg ($15.22\text{pg/mg} \pm 10.52$; mean \pm standard deviation).
214 Excluding an outlier (42.82pg/mg) the range was 1.77pg/mg to 25.22pg/mg, with a
215 mean and standard deviation of $13.38 \text{ pg /mg} \pm 7.7$. The hair cortisol of the healthy
216 dogs ranged from 1.70pg/mg to 28.79pg/mg ($17.48\text{pg/mg} \pm 8.95$). The hair cortisol
217 levels of the two groups did not differ significantly ($t = -0.655$, $df = 30$, $p = 0.517$) (the
218 outlier was included in the statistical analysis). Overall, only 18% of dogs displayed
219 chronic stress behaviours; this included 12.5% of chronically ill dogs (2 dogs) and
220 23.5% of healthy dogs (4 dogs) (see Tables 2 and 3). No significant difference was
221 found in the number of chronic stress behaviours displayed in each group ($\chi^2 =$
222 0.667 , $df = 1$, $p = 0.414$). In addition, dogs performing chronic stress behaviours did
223 not have significantly different hair cortisol concentrations to those not performing
224 them ($t = 1.377$, $df = 31$, $p = 0.179$).

225

226 **3.3 Disease-dependent factors and hair cortisol levels**

227 Of the chronically ill dogs, 62.5% (10 dogs) experienced signs of clinical disease
228 (see Table 2). However, dogs with clinical signs did not have significantly different
229 hair cortisol levels to those without them ($t = 0.321$, $df = 14$, $p = 0.753$). Eighty-one
230 per cent of chronically ill dogs (13) had lifestyle restrictions imposed by disease.
231 Dogs experiencing lifestyle restrictions did not have significantly different hair
232 cortisol concentrations to dogs not experiencing these ($t = 0.154$, $df = 14$, $p =$
233 0.880). Three ill dogs but no healthy dogs had been hospitalised within the previous
234 year. Unsurprisingly, chronically ill dogs visited the vet highly significantly more often
235 than healthy dogs ($U = 52$, $df = 31$, $p = 0.001$). However, hair cortisol levels were not
236 significantly related to the frequency of visitation ($r_s = -0.152$, $df = 31$, $p = 0.397$).

237

238

Table 2: Characteristics and hair cortisol levels of ill dogs

239

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

240 **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

241

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

244 **3.4 Disease-independent factors and hair cortisol levels**

245 There was no significant difference in the hair cortisol levels of male and female
246 dogs ($t = 1.274$, $df = 31$, $p = 0.212$). Ill dogs were highly significantly older than
247 healthy dogs (mean age of ill dogs = 9.31 years \pm 3.497 standard deviation; mean
248 age of healthy dogs = 4.63 years \pm 2.247 standard deviation; $t = 4.470$, $df = 31$, $p <$
249 0.001). However, hair cortisol levels were not significantly related to age ($r = 0.666$,
250 $df = 31$, $p = 0.714$). Chronically ill dogs were left alone for a mean time of 3.5 hours
251 (\pm 3.16 standard deviation) and the entire sample group was left alone for a mean
252 time of 3.7 hours (\pm 2.87 standard deviation). There was no significant correlation
253 between the hair cortisol concentrations of chronically ill dogs and the length of time
254 they were regularly left alone ($r_s = 0.276$, $df = 14$, $p = 0.30$). However, when the
255 subject groups were considered together (both healthy and chronically ill subjects),
256 hair cortisol levels were significantly and positively related to the length of time
257 regularly left alone ($r_s = 0.417$, $df = 31$, $p = 0.016$). In contrast, time regularly left
258 alone could not significantly predict the presence of chronic stress behaviours (odds
259 ratio = 0.862, $p = 0.416$). No significant difference was found in the hair cortisol
260 levels of dogs from single or multiple dog households ($t = -0.803$, $df = 31$, $p = 0.428$)
261 and hair cortisol levels were not significantly correlated with time regularly left alone
262 in either single ($r_s = 0.452$, $df = 12$, $p = 0.104$) or multidog households ($r_s = 0.400$, df
263 $= 17$, $p = 0.089$). However, the hair cortisol levels of healthy dogs were significantly
264 correlated with time regularly left alone in single ($r_s = 0.726$, $df = 7$, $p = 0.027$), but
265 not multidog households ($r_s = 0.528$, $df = 6$, $p = 0.179$).

266

267 **4. Discussion**

268 **4.1 Hair colour and cortisol levels**

269 In this study, hair cortisol concentrations did not significantly differ between black-
270 haired and non-black-haired subjects. This contrasts with the results of Bennett and
271 Hayssen (2010), who found that the hair cortisol levels of black dogs were
272 significantly lower than those of non-black dogs. However, Bennett and Hayssen
273 (2010) only studied German shepherd dogs and Labrador retrievers, while our
274 subject group contained a greater variety of breeds, including crossbreeds. Our
275 finding may simplify the experimental design of future research, as dogs with black
276 hair may not need to be considered separately to those with non-black hair.

278 **4.2 Chronic stress state of subjects**

279 This research found that the hair cortisol levels of healthy and chronically ill dogs did
280 not differ significantly. Chronic stress behaviours were only performed by a small
281 number of dogs and there was no significant difference in their prevalence in each
282 group. These results suggest that either both groups were chronically stressed or
283 that neither were chronically stressed. The lack of a normal range of canine hair
284 cortisol, the current scarcity of published research applying this technique, and the
285 lack of standardisation in hair cortisol extraction and assay methods, create
286 difficulties for interpretation. However imperfect, comparison to other studies is
287 presently the only aid to interpretation. Bennett and Hayssen (2010) used similar
288 cortisol extraction and assay techniques as the present study and had results similar
289 to our findings [Bennett and Hayssen, 2010; old hair growth; 12.63 ± 5.45 pg/mg
290 mean \pm standard deviation; present study; 15.22 pg/mg ± 10.52 ; mean \pm standard
291 deviation (ill), 17.48 pg/mg ± 8.95 (healthy)]. Although the health status of their
292 subject dogs was not disclosed, their sample group were living in a home
293 environment and the majority were not exposed to any major stressors (with the
294 potential exception of 2 dogs who had recently weaned puppies and 2 dogs who
295 were guide dogs). Accorsi *et al.* (2008) found a much lower mean hair cortisol
296 concentration in dogs (2.10 ± 0.22 pg/mg). However, their hair samples were
297 minced, rather than powdered, and this may result in lower cortisol extraction yields
298 (Bennett and Hayssen, 2010). In addition, they used a radioimmunoassay rather
299 than an enzyme immunoassay and this too may account for the disparity in results
300 (Bennett and Hayssen, 2010). Although Siniscalchi *et al.* (2013) used the same
301 cortisol extraction and assay techniques as Accorsi *et al.* (2008) they found much
302 higher hair cortisol levels (10:00 hour = 65.53 pg/mg + 21.49 mean + standard error;
303 17:00 hour = 96.01 pg/mg + 9.57; originally presented in pM/g). Indeed their results
304 were also much higher than those of Bennett and Hayssen (2010), and those of the
305 current study. However, Siniscalchi *et al.* (2013) exposed their subjects to various
306 acoustic stimuli, including noise from a simulated thunderstorm, and hair cortisol
307 was measured two weeks later. At 9 am hair cortisol levels were significantly and
308 positively correlated with acute stress behaviours displayed during stimuli
309 presentation, likely reflecting a state of chronic stress caused by the sounds
310 (Siniscalchi *et al.*, 2013). As the hair cortisol levels from dogs in a normal home
311 environment (Bennett and Hayssen, 2010) were similar to those in our study and as
312 the levels of stressed dogs (Siniscalchi *et al.*, 2013) were much higher, we may

313 deduce that the subjects in our research were not chronically stressed. The absence
314 of a chronic stress response to on-going disease is curious given that Church *et al.*
315 (1994) and Kaplan *et al.* (1995) found significantly higher cortisol levels in ill dogs
316 compared to healthy dogs. However, the cortisol sampling methods employed by
317 these studies (plasma cortisol, Church *et al.*, 1994; urinary cortisol/creatinine ratio,
318 Kaplan *et al.*, 1995) identify acute stress but not chronic stress (Davenport *et al.*,
319 2006). Also, neither research group distinguished acutely ill from chronically ill dogs
320 for the purposes of testing (Church *et al.*, 1994; Kaplan *et al.*, 1995). One of our
321 additional findings was that dogs performing chronic stress behaviours did not have
322 significantly higher hair cortisol levels than dogs not performing any. The concept of
323 animal “coping styles” (Koolhaas *et al.*, 1999) may provide an explanation for this.
324 Animals with proactive coping styles mount a sympathetic response to stress and
325 are likely to react actively to stressors (Koolhaas *et al.*, 1999). This may predispose
326 them to the development of stereotypies (Koolhaas *et al.*, 1999). In contrast,
327 animals with reactive coping styles are likely to mount a cortisol response and avoid
328 or withdraw from a stressor (Koolhaas *et al.*, 1999).

329

330 **4.3 Disease-dependent factors and chronic stress**

331 A variety of diseases were included in our study (see Table 2). These included both
332 progressive and nonprogressive diseases, of inflammatory and noninflammatory
333 origin. Inflammatory diseases may directly trigger a stress response via the release
334 of mediators such as cytokines (O’Connor *et al.*, 2000). However, the consequences
335 of any disease type may stimulate a stress response if negatively perceived by the
336 animal (Ursin and Eriksen, 2004). In humans, it has been observed that individuals
337 with chronic pain have significantly higher hair cortisol levels and perceived stress
338 scores than pain-free controls (Van Uum *et al.*, 2008). In addition, nausea was
339 significantly correlated with perceived stress levels in pregnant women (Chou *et al.*,
340 2008). However, in our research, ill dogs with clinical signs did not have significantly
341 different hair cortisol levels to those without clinical signs. It is possible that some
342 clinical signs (such as pain, vomiting or dyspnoea) may be inherently more stressful
343 than others (such as polyuria and polydipsia), or that clinical signs are acutely or
344 intermittently stressful. Alternatively, our small sample size may have influenced our
345 results. In addition, some signs reported as being disease related may actually be
346 side effects attributable to the patient’s medication (for example; polyuria and
347 polydipsia may be caused by diuretics administered in cardiac failure). However,
348 these too could cause stress if negatively perceived. As a discrepancy between an

349 animal's environment and its expectations may stimulate stress (Dantzer and
350 Mormède, 1983; Ursin and Eriksen, 2004), one might also expect on-going lifestyle
351 restrictions to be a source of chronic stress. However, the hair cortisol
352 concentrations of dogs with lifestyle restrictions were not significantly higher than
353 those without them. Once again, however, our sample size may have affected our
354 results. Disease severity (including severity of clinical signs and lifestyle restrictions)
355 may be an important factor to consider, as Kaplan *et al.* (1995) observed that dogs
356 with severe disease had significantly higher serum cortisol levels than dogs with
357 mild to moderate disease. Our research did not specifically investigate this, because
358 of the difficulties involved in gathering data for this purpose in multiple disease
359 types. However, we did notice that one subject had a particularly high hair cortisol
360 level (42.82 pg/mg). This subject was diagnosed with osteoarthritis and locally
361 invasive anal neoplasia (possibly adenocarcinoma), on the basis of physical
362 examination. His high hair cortisol concentration could be explained by the severity
363 of his diseases and/or the presence of multiple disease types. Future research could
364 investigate the relationship between disease severity and canine chronic stress and
365 this may be simplified by focusing on single disease states. As none of our healthy
366 subjects had been hospitalised within the previous year we were unable to study its
367 effect on hair cortisol concentrations. However, we did examine the effect of visiting
368 the veterinary clinic. Given that 78.5% of dogs display fearful behaviour upon visiting
369 the veterinary clinic (Döring *et al.*, 2009), it was surprising that in our subjects, hair
370 cortisol was not significantly related to the number of trips made to the veterinarian
371 during the previous year. This may be explained by habituation or by the triggering
372 of only an acute stress response. Alternatively, it is likely that the samples collected
373 were not representative of a full year's hair growth.

374

375 **4.4 Disease-independent factors and chronic stress**

376 In our research we also investigated the effects of disease-independent variables on
377 chronic stress levels. These included; age, gender, time left alone and the effect of
378 living in a single or multidog household. Although our chronically ill subjects were
379 highly significantly older than our healthy subjects, hair cortisol was not significantly
380 related to age. This is in agreement with Palazzolo and Quadri (1987), who found no
381 significant difference in the mean plasma cortisol levels of adult and old dogs.
382 Although gender is not itself a stressor, it may influence stressor perception. Beerda
383 *et al.* (1999b) observed that bitches had greater stress sensitivity than dogs, as
384 demonstrated by an increased salivary cortisol response to a sound blast and higher

385 cortisol induction by corticotrophin releasing hormone. Therefore, gender could
386 ameliorate or enhance the effect of a disease-related or independent stressor.
387 However, this hypothesis could not be confirmed by the current research, as there
388 was no significant difference in the hair cortisol levels of male and female dogs.
389 Rehn and Keeling (2011) observed that canine greeting behaviour intensified with
390 an increasing length of owner absence, but were unable to attribute this to distress
391 caused by separation. Within our research, the hair cortisol levels of chronically ill
392 dogs were not significantly related to time regularly left alone. However, this was a
393 small sample group. When healthy dogs were considered in addition to chronically ill
394 dogs, hair cortisol levels were significantly and positively correlated with the length
395 of time regularly left alone. However, time regularly left alone could not significantly
396 predict the presence of chronic stress behaviours. To our best knowledge, this is the
397 first indicator that canine chronic stress levels may be affected by the duration of
398 owner absence. Because time regularly left alone accounts for only 17% of the
399 variability in hair cortisol, this should be interpreted with caution and future research
400 may be required to confirm this finding. Previous research found significantly higher
401 stress levels (Bennett and Hayssen, 2010; hair cortisol levels), or a tendency
402 towards this (Dreschel and Granger, 2005; salivary cortisol levels), in multidog
403 households compared to single dog households. In contrast, we did not find a
404 significant difference in the hair cortisol levels of dogs from single or multidog
405 households. Moreover, we identified a significant and positive interaction between
406 time regularly left alone and the hair cortisol levels of healthy dogs in single dog but
407 not multidog households. Canine relationships can be complex and it is possible
408 that there was low social stress within our multidog households. The company of
409 other dogs may also act as a buffer against the stress of being left alone. In the
410 study of Dreschel and Granger (2005), the salivary cortisol levels of dogs from
411 multidog households increased significantly less than those from single dog
412 households after exposure to a recorded thunderstorm. However, owing to our small
413 sample size and the lack of a similar finding in our pooled subject groups, it is
414 difficult to interpret the significance of this result. Nonetheless, it would seem
415 sensible to minimise time regularly left alone, especially if the dog is living in a single
416 dog household, as chronic stress may be detrimental to health (Dreschel, 2010;
417 McEwen, 2005).

418

419 **5. Conclusions**

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

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448

449 **Ethical considerations**

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

452

453 **Conflict of Interest statement**

454 The authors declare that there is no known conflict of interest associated with this
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457

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