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Evaluating pair versus solitary housing in kennelled domestic dogs (*Canis familiaris*) using behaviour and hair cortisol: a pilot study

Emma K Grigg,[1,2] Belle Marie Nibblett,[1] James Q Robinson,[1] Judit E Smits[3]

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[1]Clinical Sciences, Ross University School of Veterinary Medicine, Basseterre, West Farm, Saint Kitts and Nevis
[2]School of Veterinary Medicine, University of California, Davis, CA 95616, USA
[3]Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta T2N 1N4, Canada

Correspondence to
Emma K Grigg; ekgrigg@gmail.com

ABSTRACT

Numerous studies conducted to assess welfare of domestic dogs housed in kennel facilities have reported that these dogs experience suboptimal living conditions. One important goal of improving welfare of kennelled dogs is to reduce their stress levels, and one recommended approach for improving welfare of kennelled dogs is group or social housing. The beneficial effects of management changes designed to achieve this goal should be measurable in individual animals. Stress is evident through behaviours exhibited, as well as via the concentration of cortisol, a key hormone reflecting stress. Using behavioural and hair cortisol measures, we conducted a pilot study to measure the impact of switching dogs housed in a long-term kennels facility from solitary to pair housing, using both within-subjects and between-groups comparisons. Considerable individual variation in dog responses was noted, with only two of eight pair-housed dogs showing significant declines in multiple stress-related behaviours once in pair housing. The most sensitive behaviours were active vigilance and repetitive movements (such as jumping and pacing). Barking was reduced overall in the facility following the housing change, even among dogs still in solitary housing. The long-term stress as reflected in hormone deposition in hair also provided encouraging indications that the dogs experienced lower stress levels when in paired housing; dogs showed a significant decline in hair cortisol levels from the first (prehousing change) to second (postintervention) samples. Domestic dogs are social animals, and numerous indications of potential benefit were recorded with no negative impacts seen. Based on our findings, we recommend pair or group housing of compatible dogs as a promising addition to the strategies available to those seeking to improve welfare of kennelled dogs. Future studies using higher numbers of animals and that include tracking of hair cortisol, vigilance behaviour, repetitive movements and barking would be desirable.

INTRODUCTION

It is well documented that captive animals experience stress (eg, Beerda and others 2000, Markowitz and Woodworth 1978, Morris and others 2011), and a goal of optimal welfare should be to minimise stress. As we work towards this goal by changing the social or physical environment, the effect of the change should be reflected by the animal; it is important therefore to establish meaningful, measurable outcomes of any inputs designed to increase welfare (Maple and Perdue 2013). Stress is evident through behaviours, as well as via the concentration of the main stress hormone, cortisol. Numerous studies conducted to assess welfare of domestic dogs (*Canis familiaris*) housed in kennel facilities have reported that dogs housed in kennels (particularly for longer periods of time) experience suboptimal living conditions (eg, Hubrecht and others 1992, Beerda and others 1999, 2000, Stephen and Ledger 2005). Studies of kennelled dogs have used behavioural, physical, physiological and (more recently) cognitive measures of welfare (see Hewson and others (2007) for a review; also Titulaer and others (2013) for more recent work). Welfare of these dogs may be compromised due to numerous factors: lack of exercise and/or control over their environment, confinement to a small area, minimal social contact (Hetts and others 1992, Hennessy 2013, Rooney and others 2009), novelty of the environment (Tuber and others 1996, Tuber and others 1996), high and/or unpredictable noise levels and disrupted routines (Beerda and others 1997, Hennessy 2013, Hubrecht and others 1992). Activation of the hypothalamic–pituitary–adrenal (HPA) axis indicates that dogs experience acute stress following admission to kennels, and some experience chronic stress when kennelled long term (Beerda and others 2000, Rooney and others 2007). Acute stress has been measured using cortisol levels (the end product of the HPA axis) in serum, saliva, urine (specifically as the cortisol/creatinine ratio) and faeces (eg, Beerda and others 2000, Titulaer and others 2013, Hennessy 2013, Part and others 2014).

Chronic stress levels in dogs have more recently been assessed using cortisol in hair

samples (Bryan and others 2013, Siniscalchi and others 2013, Roth and others 2016). Salivary and faecal cortisol measures provide information about stress experienced in the preceding minutes (saliva) or hours (faeces). Therefore, to monitor chronic stress from less desirable social or physical environments using faeces or saliva, a higher number of samples must be collected at multiple time periods. Hair integrates cortisol and other steroids over the entire period of hair growth, thus representing stress and reproductive activity over the preceding weeks to months (Sheriff and others 2011, Bryan and others 2013). Measurement of cortisol immunoreactivity in hair is therefore both practical and meaningful, as it is less invasive than serum and salivary cortisol measurements (Bennett and Hayssen 2010), more indicative of long-term or chronic stressors than are cortisol in serum, faeces or saliva, and less labour-intensive than repeated salivary or faecal measurements (Russell and others 2012). Further, because of the impact on diurnal rhythm of cortisol release in the body on acute stress measures (such as salivary and serum sampling), cortisol as measured in hair is the most meaningful measure of chronic stress in animals (Bryan and others 2013). Hair cortisol analysis has been used, for example, to assess stress associated with a major housing change in rhesus macaques (Davenport and others 2006). In addition, cortisol levels in hair are less variable between individuals than cortisol in saliva, faeces, etc (Bennett and Hayssen 2010, Bryan and others 2013). Bennett and Hayssen (2010) reported positive correlations between hair and salivary cortisol samples, although this finding was not supported by Bryan and others (2013). Importantly for the value of this new methodology, Bennett and Hayssen (2010) reported no significant effects of age, breed, weight or neuter status on hair cortisol, which supports hair cortisol as a useful tool for comparisons of individual traits such as resilience to chronic stress.

Numerous authors have noted that dogs vary in their individual responses to stress, behaviourally and physiologically (eg, Hiby and others 2006, Bennett and Hayssen 2010, Titulaer and others 2013, Part and others 2014). However, it is generally agreed that maladaptive and repetitive behaviours such as self-mutilation and stereotypies (defined as 'repetitive, invariant behaviour patterns with no obvious goal or function'; Mason 1991) are indicative of chronic stress (Beerda and others 2000, Hewson and others 2007). In addition to physiological changes (eg, in cortisol levels), other changes commonly associated with chronic stress of kennel life include indications of frustration (such as chewing, vocalising), conflict (body-shaking, paw-lifting), coprophagy (Beerda and others 1997) and a lowered/fearful posture (Hewson and others 2007). Other behavioural changes, such as increased or decreased activity levels, vary by dog and by study (eg, Hubrecht and others 1992, Hetts and others 1992, Beerda and others 2000). Most studies report increased activity in dogs living in suboptimal housing conditions, likely associated with repetitive behaviours; reports of reduced activity in dogs living in suboptimal conditions may reflect fatigue or boredom (as suggested by Beerda and others 1997) or learned helplessness. Apparent over-reaction by kennelled dogs to relatively mild stimuli, often triggering repetitive behaviours such as circling and jumping, has also been documented (Beerda and others 2000, Hewson and others 2007), perhaps due to the dog's frustration at not being able to reach and interact with the stimulus. Chronic stress has negative impacts on the overall health and wellbeing of kennelled dogs (Hennessy 2013). Because of individual variation among dogs, facilities and methods used to assess responses to interventions designed to improve welfare of kennelled dogs, many authors have suggested using multiple measurements (ie, physiological and behavioural) and an integrated analytical approach (Beerda and others 2000, Hiby and others 2006, Titulaer and others 2013).

One frequently cited concern about welfare of dogs living in kennelling facilities is solitary housing. Although kennel size is an often-cited concern, Hetts and others (1992) note that social isolation may be equally or more harmful than spatial restriction. Group housing is a suitable alternative from a welfare perspective, providing opportunities for positive interaction with other animals including play, companionship, physical connection and socialisation. Mertens and Unshelm (1996) report in a study of 211 shelter dogs that a high percentage of dogs housed alone suffered from behavioural problems (31 per cent), with 10 per cent developing stereotypies. They also found that increased aggression in group-housed dogs, a frequently cited reason to house dogs singly, was not seen in their study. Ninety-one per cent of social confrontations between dogs in the Mertens and Unshelm (1996) study were settled without actual physical conflicts. Hubrecht and others (1992) also noted that dogs differed greatly in their behaviour when housed singly versus in groups: dogs housed alone were more inactive (72–85 percent of time v 54–62 per cent in group-housed dogs) and spent more time in non-social repetitive behaviours like circling (4–5 per cent of time compared with 0.9–2 per cent in group-housed dogs). Group housing can be used to provide a more enriched and varied environment (ASV 2010), and providing dogs with increased social contacts may enable a dog to gain more control over his/her environment. Actual or even perceived control over one's environment is an important aspect of quality of life, which may in turn increase the dogs' ability to cope with the pressures of confinement (Hubrecht and others 1992). The Center for Shelter Dogs (Tufts University Cummings School of Veterinary Medicine, Medford, Massachusetts, USA) states that 'co-housing dogs must be a consideration for dogs kept longer than two weeks' (Center for Shelter Dogs online, accessed June 2015). There is no single accepted definition of what constitutes 'long-term housing' for kennelled dogs, with studies reporting increased occurrence of chronic stress-related behavioural issues in as little as 4–8 weeks following admission to a kennels facility (eg,

Beerda and others 1999, Stephen and Ledger 2005). For this study, we consider 'long-term' to be any stay longer than six months.

In the present study, our goal was to assess the impact of switching dogs housed in a long-term kennel facility from solitary to compatible pair housing, using multiple measures (behavioural and hair cortisol), and considering within-subjects and between-subjects comparisons. Due to logistical constraints, we consider this a pilot study that can provide baseline information for future work. Our hypotheses were that (1) there would be a measurable reduction in physiological and behavioural stress indicators in dogs housed in compatible pairs, relative to dogs in the same facility remaining in solitary housing for the same time period; and (2) hair cortisol results would reflect beneficial effects similar to those reported in earlier studies of group housing of kennelled dogs.

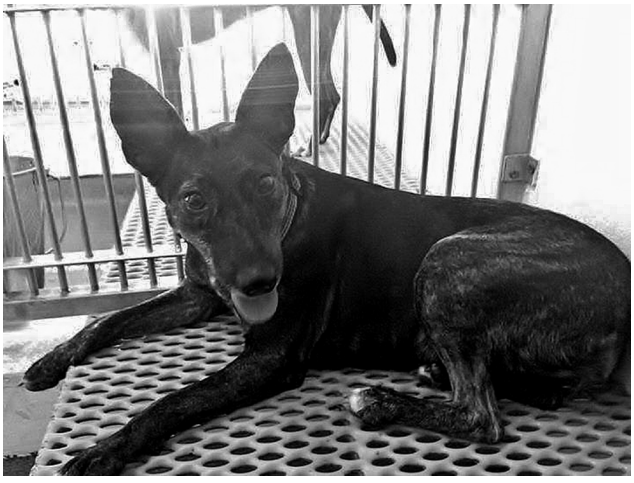

FIG 1: Study dog (Paired 1B) inside one of the individual kennels in this study, showing side wall grates allowing visual and limited interaction with neighbouring dogs. (Photo: P Turmenne).

MATERIALS AND METHODS

Study population

The study was conducted at Ross University School of Veterinary Medicine (RUSVM) in St Kitts, West Indies. RUSVM houses a colony of mixed-breed domestic dogs used in teaching veterinary students techniques such as conducting physical exams, according to protocols approved by the RUSVM Institutional Animal Care and Use Committee (IACUC). The colony ranges in size between 20 and 30 dogs; size of individual dogs varies but most are of medium build (15–20 kg). Dogs are accepted as donations to the programme as needed for teaching purposes. Most dogs in the colony were found as strays, or surrendered by local owners who could no longer care for them. All dogs are behaviourally assessed prior to acceptance into the colony, and dogs displaying aggressive tendencies during assessment or during the two-week to three-week quarantine period are not added to the colony. Dogs in the colony are individually housed in kennels for up to 24 months prior to adoption into suitable homes. Individual kennels are 2.36 m long, 1.07 m wide and 2.24 m high, with grates on both sides and the door allowing visual contact and limited (non-contact) interaction with neighbouring dogs and passing dogs, kennel staff and students (fig 1). Dogs are fed once per day and walked outdoors twice per day (for 15–30 minutes per walk), and kennels are cleaned once per day; these activities are done at consistent times each day. There is an active enrichment programme whereby dogs are taken out regularly (a minimum of two times per week) for training and/or socialising with conspecifics or human companions, in addition to their duties in scheduled teaching labs (one to three labs per week). Nonetheless, while in the kennels some of these dogs develop stereotypies indicative of chronic stress (Grigg and Piehler 2015). The facility management agreed to the conversion of four sets of kennels into shared housing, which would allow eight dogs to be placed in pair housing. Twelve healthy mixed-breed dogs, ranging in age between 1.8 and 4 years, and who had been residing in the colony for a minimum of six months (range: 8–20 months; mean ± se: 12.2±1.13 months) were included in the study. From these 12, eight dogs were randomly selected for pair housing; the remaining four dogs were left in solitary housing throughout the study. Seven (58 per cent) of the dogs were female, five (42 per cent) were male and eight of the 12 (67 per cent) were neutered at the time of the study (table 1). This resulted in an imbalance in the ratios of intact:altered dogs between our treatment versus control groups. However, given the lack of significant effect of age or neuter status on hair cortisol (Bennett and Hayssen 2010), and the fact that all dogs entering the kennels facility had passed an initial behavioural assessment (ruling out dogs with elevated levels of aggression), we felt that this imbalance would not represent a significant confounding variable in these comparisons.

For safety and to avoid inadvertently increasing the stress of individual dogs, it is important to consider compatibility between dogs when moving them into shared housing (Wells 2004). The four pairs randomly selected for cohousing were evaluated for compatibility (ie, with their potential kennel-mate) prior to placement in pair housing, using an RUSVM protocol to assess suitability of dogs for shared housing (box 1). This study was conducted with approval from the RUSVM IACUC (protocol #13–031).

Data collection (behavioural)

All 12 dogs were videotaped in their solitary kennels for 30 minutes, twice per week at the same time each day, for two weeks prior to the transition of eight of the dogs to pair housing (and prior to the possible confounding stressors of construction work to convert the existing single kennels to shared/pair kennels). Timing of video recording was selected to avoid predictable disruptions to the dogs' daily routine, such as student walking, feeding and kennel cleaning, and video data for this

TABLE 1: Dogs included in the pair-housing study

Dog ID	Dog name	Sex	Reproductive status	Age (years)*	Length of stay (months) at first hair collection
Paired 1A	Nike	Male	Neutered	1.8	12
Paired 1B	Selly	Female	Spayed	2	10
Paired 2A	Thunder	Female	Spayed	2	9
Paired 2B	Snow	Female	Spayed	2	13
Paired 3A	Georgia	Female	Spayed	2	14
Paired 3B†	Clove	Female	Spayed	4	16
Paired 4A	Kato	Male	Intact	1.8	8
Paired 4B	Tucker	Male	Neutered	2	10
Solitary 1	Mystic	Male	Intact	2	9
Solitary 2	Lucy	Female	Intact	3	17
Solitary 3	Lilly	Female	Intact	4	8
Solitary 4	Bumper	Male	Neutered	2	20

All dogs were housed in solitary kennels during the baseline study period; experimental dogs were pair-housed and control dogs remained solitary during the treatment period. Pairs are identified according to the dog they were paired with, once moved into pair housing (eg, dogs 'Pair1A' and 'Pair1B' were housed together after the change).
*Exact age is rarely known for colony dogs (except for dogs 'Paired 1A' and 'Paired 4A', who were born onsite); age is estimated by the veterinary staff when the dog is first added to the colony.
†This dog was being treated for anxiety with the tricyclic antidepressant amitriptyline (50 mg orally, twice daily) before the study commenced and for the duration of the study.

pilot study was collected at consistent times each day in order to minimise any additional variation in behaviours associated with time of day. GoPro Hero3 (GoPro, San Mateo, California, USA) cameras were set up on the kennel opposite the focal (subject) dog's kennel, after which the researcher left the area to avoid influencing the dog's behaviour. Following baseline data collection, eight individual kennels were converted into four shared/pair kennels, by installing a guillotine-style door in the concrete wall between two adjacent kennels, and allowing dogs to interact freely with each other during daylight hours. This design had the benefit of maintaining the same average kennel space per dog, to reduce any confounding effects of an increase in kennel space on behaviour and cortisol. The dogs were separated and the guillotine door closed during their once-daily feeding and at night when no kennel staff were on site. Following completion of the conversion construction and a one-week 'rest' period postconstruction, eight dogs (hereafter 'experimental dogs') were moved into their new shared housing, and video data collection was resumed for eight weeks on all 12 dogs (eight now in shared housing, four remaining in solitary housing). The four dogs that remained in solitary housing ('control dogs') were used as environmental controls, providing information on behavioural effects of any ambient changes distinct from shared housing, which could have confounded the interpretation of results.

Box 1 Ross University School of Veterinary Medicine Protocol (2014) for assessing dogs for pair housing (adapted from a draft protocol written by R Hack, RUSVM)

- Only trained kennels personnel familiar with the dogs under consideration should conduct the assessment sessions and make the decision on whether the dogs would be suitable as 'living pairs'.
- In order to be considered suitable for pair housing, the dogs should have had 5 plus interactions of 30 minutes or more during which there were no aversive behaviours seen (examples of aversive behaviours include overtly aggressive body language (threats), avoidance of the other dog or guarding behaviour).
- Neither dog should display guarding behaviour in any form (toys, food, favourite resting spot, etc) in the presence of each other at any point during their interactions.
- Both dogs should have similar energy levels.
- When playing the dogs should have periods of calm activity (not playing constantly). This may not be seen in the first 15-20 minutes after meeting, but there should be periods of calm after the initial excitement has worn off.
- At least one of the dogs should be neutered in any 'living pair'.
- For opposite sex pairings the male should not mount and tie with the female at any point during their interactions.
- Once a 'living pair' has been identified, the pairing must be approved by the clinician in charge of the kennel programme and the kennels facility manager.

From the video footage, we recorded occurrence and duration of the following behaviours, based on the literature on behaviours indicative of stress in kennelled dogs: repetitive vocalisation (barking), circling, spinning, pacing, barrier jumping and conflict-related behaviours, as well as time spent resting (lying down and/or sleeping). To allow for the dogs to adjust to the researcher leaving the kennels area, we began data recording after 1 minute of video had passed. Footage when the dogs were out of view was removed from the analysis. We used focal animal sampling (Altmann 1974) and recorded behaviour in two ways: continuous (in which start and end times of all behaviours were recorded and considered 'duration' of behaviours) and instantaneous scan sampling (in which behaviour and body posture of the focal dog were recorded at 1-minute intervals). For continuous sampling, the following behaviours were recorded: barking, jumping, pacing (repetitive movement around kennel), resting/sleeping, spinning (ie, repetitive, tight circles), vigilance (when dog was alert and actively looking at something outside his/her kennel) or 'other' (with specific behaviour noted). For instantaneous sampling, the following variables were recorded at each 1-minute scan: body posture (sitting, standing or lying down); whether or not the dog was actively vigilant or barking; movement type (none, jumping, pacing, spinning or directional movement from one part of the kennel to another); and any social behaviour. Examples of social behaviour for cohoused dogs consisted of none, in physical contact with, playing with or exhibiting aggression towards another dog in a directly adjacent kennel or in the shared kennel. Examples for solitary dogs consisted of none, any affiliative or any aggressive interaction with a dog in a directly adjacent kennel (fig 1).

Data collection (hair cortisol)

A 10 cm × 10 cm patch of hair was collected from the shoulder of all dogs using hand-held electric clippers with a number 10 blade, at two times: prior to the start of the kennels construction work (for baseline cortisol levels) and after the dogs had been in the shared/pair housing for eight weeks (for postintervention levels). Three days after being moved into the pair housing, the patch was reshaved on all dogs, so that hair growth in the second sample reflected stress levels during the treatment period (when the paired dogs were cohoused).

Sample analysis (hair cortisol)

We used a commercial enzyme immunoassay kit (Salimetrics, Philadelphia, Pennsylvania, USA), previously validated in our laboratory (Bryan and others 2013), to quantify cortisol in hair as described below. Hair samples were processed as described in Bryan and others (2013) with the following modifications. After distilled water and isopropanol washes, the dried hair samples were powdered in a ball mill (Mixer Mill 200, Retsch, Haan, Germany), then 20 mg was weighed into a 20 ml glass scintillation vial. High Performance Liquid Chromatography (HPLC)-grade methanol, 2.5 ml, was added to each vial. Samples were sonicated, extracted and centrifuged for 15 minutes at 14 000 *g*. A 0.31 ml aliquot was removed and dried under a gentle stream of nitrogen. Samples were reconstituted in 720 µl of 5 per cent methanol followed by 95 per cent assay diluent. Each sample, in duplicate, was assayed according to kit instructions.

The intra-assay coefficients of variation for high and low kit standards were 3.6 per cent and 7.3 per cent, respectively. The interassay coefficients of variation for the high and low kit standards, based on two validation assays, were 2.5 per cent and 13.2 per cent. The extraction efficiency was 98.5 per cent based on triplicate hair samples spiked with 162 pg of cortisol prior to the extraction and processing described above, and compared with three replicates spiked after extraction.

Statistical analyses (behavioural)

Individual dogs will vary in their behavioural baselines due to temperament, age, sex and past experience. We used a within-subjects comparison to look for significant differences in each dog's behaviour before and after being moved from individual to pair housing. The still solitary, control dogs were similarly evaluated. We also used a between-groups comparison of per cent change in time spent in a given behaviour, from the baseline (all dogs housed in solitary kennels) to treatment (experimental dogs housed in paired kennels, control dogs remaining in solitary kennels) periods.

Within-subjects (continuous data)

For the continuous video data, duration in minutes of specific behaviours during each video session was converted into the proportion of time spent in that behaviour for that session. For each dog and each behaviour, the mean proportion of time spent in that behaviour was calculated for the two time periods, baseline and treatment. Wilcoxon signed-rank tests were then used to compare the relative amount of time spent engaged in each behaviour, before versus after the housing change was made for (1) the experimental (pair-housed) dogs and (2) the control (solitary) dogs.

In addition, we ran a repeated-measures analysis of variance (ANOVA) to compare baseline versus treatment period behaviours for all 12 dogs simultaneously, with housing type (paired or solitary) and time period (baseline and treatment) as fixed effects, which allowed us to also assess any interaction effects between dog group and time period. The repeated-measures ANOVA was run on the mean proportion of time each dog spent in a given behaviour during each of the two time periods. Prior to the ANOVA analysis, a log transformation was used on the behavioural proportions to normalise these data; residuals were tested for normality using the Shapiro-Wilk statistic prior to interpretation of the ANOVA.

Within-subjects (instantaneous data)

To increase statistical independence of scan sample intervals, we followed Bernstein's (1991) recommendation so that intervals between scans exceeded the mean duration of each specific behaviour by at least 1.96 sd. For the instantaneous data analysis, therefore, we used scans at 3-minute intervals, and not the 1-minute intervals originally recorded. For each behavioural category (eg, motion), the number of scans noting a particular behavioural state (eg, moving from one part of the kennel to another, jumping, pacing, etc) was tabulated for each dog. Some behavioural states were rarely or never recorded in the scan data, so data were pooled into four broader categories for analysis: barking, in motion, socialising and vigilance. The number of scans spent in a given behavioural category was then converted into the relative proportion of all scans collected for that session. For each dog and each behavioural category, the mean proportion of scans spent in that behaviour was calculated for the two time periods, baseline and treatment. Wilcoxon signed-rank tests were then used to compare the proportion of scans spent in a given behaviour in the baseline versus treatment periods for (1) the experimental dogs and (2) the control dogs.

Between-groups comparison of behavioural change (continuous data)

Finally, we used a Mann-Whitney U test to compare the per cent change in mean time spent in a given behaviour (ie, from the baseline to treatment periods) in the control dogs versus in the experimental dogs. This between-groups analysis was done to determine whether there was a significant difference in the magnitude of change between the two groups. This analysis was run only for behaviours for which, in the results of the Wilcoxon signed-rank analyses, the probability of seeing the observed changes from the baseline to treatment periods by chance alone was ≤0.10.

Statistical analyses (hair cortisol)

We compared hair cortisol levels in the first (baseline) versus second (post-treatment) samples for the pair-housed (experimental) dogs and for the solitary (control) dogs, using Student's *t* tests for paired samples. We also calculated the per cent change in cortisol from baseline to post-treatment period levels for the eight pair-housed and the four solitary dogs. To determine whether greater changes were seen in the pair-housed dogs than the solitary dogs (our original hypothesis), we then compared the relative changes in cortisol levels in the pair-housed versus solitary dogs using a two-tailed Student's *t* test. Cortisol data were assessed with Shapiro-Wilk tests for normality prior to running the Student's *t* tests.

All statistical analyses were done in XLSTAT (Addinsoft, Paris, France) or Minitab (Minitab, State College, Pennsylvania, USA). Significance was set at α=0.05. Given our small sample size and in common with similar analyses in Part and others (2014) and Beerda and others (2000), we did not correct for multiple comparisons, as we believe that would have greatly increased our chance of committing type II errors. We did use within-subjects comparisons and two concurrent approaches (continuous and instantaneous scan sampling) to better understand the behavioural data, and we looked for instances where results from one analysis method supported the other; nonetheless, our results should be viewed with this caveat in mind.

RESULTS

Study population

All dogs selected for pair housing passed the compatibility assessment (box 1) with no aggression between pair-housed dogs.

Behavioural results

After removal of video footage during which a dog was out of view or not clearly visible due to low ambient lighting, 81 hours of video were included in the behavioural analyses.

Within-subjects (continuous data)

For the experimental dogs, slight declines were seen in the proportion of time dogs spent in stress-related behaviours, as well as in resting, from the baseline to the treatment periods (table 2A), although none were great enough to reach statistical significance. Two declines (barking and pacing) approached significance (barking: V=19, P=0.09; pacing: V=19, P=0.09). For the control dogs, no significant differences were seen between behaviours in the baseline versus treatment periods (P>0.2 for all comparisons; table 2B).

Within-subjects (instantaneous data)

Duration of most behaviours recorded in the continuous data was well under the 3-minute interval of our instantaneous scan data. However, the mean duration (plus 1.96 sd) of the postural behaviours (sitting, standing or lying down) was 12.96 minutes. For this reason, we discarded this category from the instantaneous data analysis to avoid pseudoreplication within these data. For the experimental dogs, proportion of socialising was significantly higher in the treatment period (V=0, P=0.01); this increase was not surprising given the change from solitary to paired housing (table 3A). The behavioural category motion also showed an increase during the time that the dogs were cohoused, although the increase was not statistically significant (V=3, P=0.08). Closer examination of the behaviours included in the motion category revealed that the subcategory pacing (generally associated with stress) did not increase when dogs were cohoused, but rather the dogs were observed moving from one part of the kennel to another, or engaged in movements made during play. For the control dogs, no differences were seen in the dogs' behaviour during the baseline versus treatment periods (P>0.1 for all analyses; table 3B).

TABLE 2: Mean proportion of time spent in a given behavioural state for all experimental (pair-housed) (A) and control (solitary-housed) dogs (B) in the baseline and treatment periods, and results of the Wilcoxon signed-rank analyses of proportions in the baseline versus treatment time periods

	Behaviour	n	Mean±se (baseline period)	Mean±se (treatment period)	P value
A. Experimental (pair-housed) dogs	Barking	6	0.042±0.017	0.010±0.003	0.09
	Jumping	2	0.096±0.072	0.053±0.046	0.4
	Pacing	6	0.082±0.033	0.042±0.024	0.09
	Resting	8	0.605±0.078	0.445±0.091	0.1
	Spinning	2	0.006±0.005	0.003±0.002	1.0
	Vigilance	8	0.396±0.084	0.287±0.089	0.2
B. Control (solitary) dogs	Barking	4	0.014±0.004	0.013±0.004	0.6
	Jumping	3	0.009±0.004	0.005±0.003	0.4
	Pacing	3	0.014±0.008	0.010±0.002	1.0
	Resting	4	0.788±0.025	0.822±0.041	0.6
	Spinning	2	0.002±0.001	0.001±0.0008	1.0
	Vigilance	4	0.259±0.056	0.168±0.028	0.2

The repeated-measures ANOVA did not reveal any statistically significant main or interaction effects for barking or pacing (P>0.1 for all analyses). Shapiro-Wilk tests confirmed that the log-transformed data met the requirements of the ANOVA (P>0.05 for both analyses).

Given this surprising (compared with earlier studies) lack of strong differences between the behaviour of dogs in solitary versus paired housing, and the fact that our small sample size likely dampened our ability to detect significant differences between the two groups, we then examined the behaviour of each individual dog during the baseline versus treatment time periods. This allowed assessment of any discernible patterns in the direction of change (ie, a decrease v increase in a given behaviour) for individual dogs, and whether (if present) this direction of change differed consistently between the control and the experimental dogs. We used the continuous behavioural data to calculate and compare (using a Mann-Whitney U test), for each dog, the proportion of time spent in a given behaviour during the baseline and treatment periods (experimental dogs: table 4; control dogs: table 5). Again, the study dogs exhibited significant individual variation in their behaviour. This analysis revealed the following:

- Significant decreases in multiple stress-related behaviours were seen in only two of the experimental dogs (Paired 2B, a spayed female; and Paired 4A, an intact male; table 4). One other experimental dog, a neutered male, showed one significant decline, in vigilance behaviour. No significant differences between behaviours during the baseline versus treatment periods were seen in any of the other treatment dogs.
- Barking was the only behaviour that decreased significantly in the control dogs (two males in the control group, one intact and one neutered, barked significantly less during the treatment period; table 5).
- When looking at the patterns of behaviour (ie, direction of behavioural change) of the dogs in each group:

TABLE 3: Proportion of scans recording a given behavioural category for all experimental (pair-housed) (A) and control (solitary-housed) dogs (B) in the baseline and treatment periods, and results of the Wilcoxon signed-rank analyses of proportions in the baseline versus treatment time periods

	Behaviour	n	Mean±se (baseline period)	Mean±se (treatment period)	P value
A. Experimental (pair-housed) dogs	Barking	8	0.028±0.017	0.024±0.010	0.8
	Motion	8	0.072±0.027	0.206±0.064	0.1
	Socialise	**8**	**0.000±0.000**	**0.226±0.039**	**0.01**
	Vigilance	8	0.056±0.106	0.491±0.107	0.4
B. Control (solitary-housed) dogs	Barking	4	0.009±0.005	0.017±0.007	0.6
	Motion	4	0.059±0.028	0.033±0.015	0.6
	Socialise	4	0.000±0.000	0.004±0.008	1.0
	Vigilance	4	0.262±0.037	0.179±0.035	0.1

Significant difference is shown in bold.

TABLE 4: Individual behavioural data results (proportion of time spent in a given behaviour) for all experimental (pair-housed) dogs, in the baseline and treatment periods. Statistically significant differences are shown in bold, with direction of change (increase or decrease) and p value shown.

Dog ID	Behaviour*	Mean±se (baseline)	Mean±se (treatment)	Significant difference (direction of change/P)
Paired 1A	Bark	–	–	
	Jump	–	–	
	Pace	–	–	
	Rest	0.318±0.062	0.348±0.131	
	Spin	–	–	
	Vigilance	**0.681±0.059**	**0.229±0.098**	**Decrease/0.04**
Paired 1B	Bark	–	–	
	Jump	–	–	
	Pace	0.182±0.141	0.080±0.037	
	Rest	0.426±0.181	0.210±0.078	
	Spin	–	–	
	Vigilance	0.390±0.103	0.795±0.491	
Paired 2A	Bark	0.046±0.020	0.015±0.005	
	Jump	0.168±0.054	0.098±0.032	
	Pace	0.172±0.072	0.143±0.048	
	Rest	0.351±0.092	0.451±0.086	
	Spin	0.010±0.009	0.004±0.003	
	Vigilance	0.730±0.125	0.425±0.068	
Paired 2B	**Bark**	**0.013±0.004**	**0.005±0.003**	**Decrease/0.01**
	Jump	**0.024±0.015**	**0.007±0.004**	**Decrease/0.04**
	Pace	**0.011±0.007**	**0.001±0.0007**	**Decrease/0.02**
	Rest	0.673±0.108	0.622±0.073	
	Spin	0.001±0.0006	0.001±0.0004	
	Vigilance	0.531±0.095	0.423±0.082	
Paired 3A	Bark	0.007±0.004	0.016±0.007	
	Jump	–	–	
	Pace	–	–	
	Rest	0.786±0.039	0.737±0.076	
	Spin	–	–	
	Vigilance	0.153±0.025	0.129±0.036	
Paired 3B	Bark	0.039±0.013	0.020±0.006	
	Jump	–	–	
	Pace	0.011±0.011	0.022±0.012	
	Rest	0.782±0.064	0.737±0.075	
	Spin	–	–	
	Vigilance	0.125±0.053	0.145±0.035	
Paired 4A	**Bark**	**0.123±0.093**	**0.0002±0.0001**	**Decrease/0.01**
	Jump	–	–	
	Pace	**0.097±0.039**	**0.0002±0.0001**	**Decrease/0.02**
	Rest	**0.602±0.106**	**0.002±0.001**	**Decrease/0.02**
	Spin	–	–	
	Vigilance	**0.386±0.114**	**0.005±0.003**	**Decrease/0.02**

Continued

TABLE 4: Continued

Dog ID	Behaviour*	Mean±se (baseline)	Mean±se (treatment)	Significant difference (direction of change/P)
Paired 4B	Bark	0.026±0.023	0.005±0.005	
	Jump	–	–	
	Pace	0.019±0.019	0.003±0.003	
	Rest	0.918±0.034	0.454±0.197	
	Spin	–	–	
	Vigilance	0.174±0.059	0.148±0.094	

*Behaviours not recorded for a given dog are indicated with a '–' symbol.

- Experimental dogs: Of the six experimental dogs who exhibited barking in their kennels, five (83 per cent) barked less during the treatment period, but as noted above, these differences were significant for only two dogs. All study dogs exhibited vigilance behaviour in their kennels. Six (75 per cent) of the experimental dogs exhibited less vigilance behaviour during the treatment period; two of these declines were statistically significant. Of the six experimental dogs who paced in their kennels, five (83 per cent) paced less when paired, two significantly so. One of the experimental dogs spent significantly less time resting during the treatment period.

TABLE 5: Individual behavioural data results (proportion of time spent in a given behaviour) for all control (solitary-housed) dogs in the baseline and treatment periods.

Dog ID	Behaviour*	Mean±se (baseline)	Mean±se (treatment)	Statistically significant difference (direction/P)
Solitary 1	**Bark**	**0.020±0.006**	**0.005±0.003**	**Decrease/0.01**
	Jump	0.003±0.002	0.00007±0.00001	
	Pace	0.030±0.011	0.013±0.009	
	Rest	0.729±0.079	0.827±0.089	
	Spin	0.003±0.002	0.0005±0.0005	
	Vigilance	0.330±0.087	0.217±0.078	
Solitary 2	Bark	0.003±0.002	0.015±0.003	
	Jump	0.008±0.003	0.008±0.003	
	Pace	0.007±0.007	0.009±0.004	
	Rest	0.790±0.219	0.741±0.041	
	Spin	–	–	
	Vigilance	0.337±0.101	0.212±0.037	
Solitary 3	Bark	0.013±0.013	0.023±0.017	
	Jump	–	–	
	Pace	–	–	
	Rest	0.849±0.043	0.788±0.063	
	Spin	–	–	
	Vigilance	0.095±0.034	0.104±0.028	
Solitary 4	**Bark**	**0.021±0.006**	**0.008±0.003**	**Decrease/0.03**
	Jump	0.016±0.003	0.008±0.003	
	Pace	0.006±0.009	0.007±0.009	
	Rest	**0.785±0.040**	**0.933±0.013**	**Increase/0.02**
	Spin	0.0004±0.0003	0.002±0.002	
	Vigilance	0.272±0.058	0.140±0.029	

Statistically significant differences are shown in bold, with direction of change (increase or decrease) and p value shown.
*Behaviours not recorded for a given dog are indicated with a '–' symbol.

TABLE 6: Hair cortisol levels (in pg/mg) before and after the housing change and per cent change in cortisol (first to second sample) for all dogs

Dog ID	Baseline	Postintervention	Difference (pre – post)	Per cent change
Paired 1A	883.2	269.8	613.4	69
Paired 1B	1300.8	169.0	1131.8	87
Paired 2A	2646.2	466.1	2180.2	82
Paired 2B	6617.3	2587.2	4030.1	61
Paired 3A	2863.2	440.2	2423.0	85
Paired 3B	1621.0	208.8	1412.2	87
Paired 4A	6727.2	1241.3	5485.9	82
Paired 4B	889.9	158.9	731.0	82
Solitary 1	2020.3	700.8	1319.5	65
Solitary 2	4308.0	985.4	3322.6	77
Solitary 3	5247.4	2803.2	2444.2	47
Solitary 4	2610.7	288.5	2322.2	89

- Control dogs: All the four control dogs exhibited barking in their kennels, and two (50 per cent) exhibited less barking during the treatment period; both differences were significant. Three (75 per cent) of the four control dogs also exhibited a decline in vigilance, but none significantly. Of the three control dogs who paced, two (67 per cent) paced less when the others were paired, but none of the control dog declines in pacing were statistically significant. One control dog spent significantly more time resting during the treatment period.

Between-groups comparison of behavioural change (continuous data)

The behaviours bark and pace were used to look for differences between the control versus experimental dogs in the magnitude of change in their behaviour from the baseline to treatment periods. There were no significant differences between the control versus experimental dogs in the magnitude of change for either behaviour (barking: U=8, P=0.5; pacing: U=5, P=0.4).

Hair cortisol

Hair cortisol levels (baseline and postintervention periods, in pg/mg) are shown in table 6, along with per cent change in cortisol for all dogs. Average baseline hair cortisol (mean±se) for all dogs was 3144.6±605.9 pg/mg. Average postintervention hair cortisol for pair-housed dogs was 693±298 pg/mg; for solitary (non-intervention) dogs, average postintervention hair cortisol was 1195±555 pg/mg. All dogs showed a marked, statistically significant decline in hair cortisol levels from the first to second hair sample (before v after the housing change): t=3.710, df=7, P=0.008 for the pair-housed dogs, and t=5.737, df=3, P=0.01 for the solitary-housed dogs. Shapiro-Wilk tests demonstrated normality of the cortisol data (P>0.7 for both analyses). The per cent change in cortisol tended to be greater for the pair-housed (average=−79.4 ± 3.29 se) than for the individually housed (average=−69.5 ± 9.03 se) dogs, but not significantly so (P>0.2).

DISCUSSION

Although individual variation is increasingly recognised as relevant and intrinsically important in behavioural research (Koolhaas and others 1999), behavioural studies are challenging due to this considerable variation among individuals, particularly when constrained by small sample sizes. Our findings correspond with other studies noting high individual variation in canine responses to stress (both behaviourally and physiologically) and in coping strategies (Hiby and others 2006, Bennett and Hayssen 2010, Titulaer and others 2013, Part and others 2014, Miklósi and Kubinyi 2016). Given this variation, our small sample size precluded detection of consistent, statistically significant differences between our two groups of study dogs. It may be that dogs responded in different ways to the housing change, and only some of the dogs benefited significantly from the change. This explanation is supported by the comparisons of behaviour in individual dogs in the baseline versus treatment periods: significant decreases in multiple stress-related behaviours were seen in only two of the experimental dogs, while the majority of the remaining experimental dogs showed no significant changes in behaviour. Apart from a significant decline in barking in two of the control dogs, no significant changes in stress-related behaviours were seen in any of the control dogs. Interestingly, the two experimental dogs who exhibited significant declines in a number of stress-related behaviours when cohoused (ie, during the treatment period) had the two highest baseline hair cortisol measures of all 12 study dogs. The third highest baseline hair cortisol measure was recorded for one of the control dogs; this dog, housed alone during

the treatment period, exhibited no significant declines in stress-related behaviours. If future research supports this explanation for our results, then hair cortisol analyses may be useful in identifying dogs at greatest risk of negative impacts of stress (from kennelling, military service, etc), similar to the utility of hair cortisol as a biomarker for identifying humans most at risk for suffering negative health effects of stress (as suggested in Russell and others 2012).

Two alternate explanations for the lack of significant patterns exist. The first is that the dogs in this facility were simply not affected by the housing change. The between-group comparisons did not reveal consistent, statistically significant differences between the two groups, in either behaviour or hair cortisol levels. However, in light of the constraints mentioned above, and the predominance of findings in the literature indicating positive impacts of group housing, we would hesitate to conclude this based on the results of the present study. The second alternate explanation is that some other, uncontrolled variable affected the dogs' behaviour and cortisol levels during our study, clouding our ability to discern patterns due solely to the housing change. In our study, as noted by others conducting studies at working kennels operations (eg, Hubrecht and others 1992, p 380), it was not possible to control all environmental variables and standardise subjects to the degree that would normally be expected in an experimental research study. Our dogs experienced more environmental variation than those housed in controlled research laboratories, but, as a stable group of dogs with regular daily routines, less variation than dogs housed in rehoming shelters. Nonetheless, we are confident that major influences on the dogs' lives, other than the experimental manipulation, remained constant throughout our study period (baseline and treatment periods). Minor or short-lived stressors would not be reflected in hair cortisol levels (Ashley and others 2011). In addition, there are no major seasonal influences in the tropical climate of St Kitts, and no unusual events occurred in the kennels during our study period. Given these caveats, however, the results of this pilot study and the discussion that follows should be viewed with caution.

Because individuals experience and demonstrate stress in unique ways, the within-subject, individual comparisons were more informative than between-group comparisons, for both the behavioural and the cortisol analyses. Because of the inherent differences in basic 'personality' among dogs, there was marked individual variation in response to the pair housing treatment, just as one would expect differences in individuals coping with substandard living conditions. This also made it most valuable to look for patterns of change integrating both the behavioural and physiological measures.

Benefits to the dogs from moving to social, paired housing were supported by several behavioural observations. The most sensitive behaviours (significant differences seen only in the paired dogs) were vigilance and repetitive behaviours such as pacing and jumping. Active vigilance, a manifestation of stress in dogs, was recorded for shorter durations in the paired dogs during the treatment period, and there was a slight trend towards reduced time spent in repetitive behaviours (jumping, barking, pacing) in the pair-housed dogs. These changes were consistent with advantages expected based on behaviour of kennelled and shelter dogs (Hubrecht and others 1992, Mertens and Unshelm 1996, Wells 2004), in which such stress-related behaviours can be reduced by living with a conspecific. When comparing the baseline with treatment time periods, barking tended to be reduced in a number of the pair-housed dogs, some significantly so. Interestingly, two solitary dogs also decreased their barking during the treatment period. It is possible that decreased barking by the pair-housed dogs contributed to a calmer environment, suggesting an unanticipated advantage that is not directly due to being paired. This 'spill-over' effect of social barking is supported by the results of the between-groups comparison: the magnitude of the decline in barking from baseline to treatment period was similar for both groups. The consistency of the dogs' schedules and environment over the course of the study support that the behavioural impacts in our animals were due directly or indirectly to the improved social situation of the paired dogs. Nonetheless, we strongly recommend that future similar studies be carried out with a considerably larger sample size.

Although individual dogs vary in their tolerance for kennel conditions, social interactions between the pair-housed dogs in our study were overwhelmingly positive. The primary source of past resistance to pair housing of dogs was fear of aggression and injury to dogs and/or kennel staff, but our findings corroborate those of Mertens and Unshelm (1996), who found these concerns were largely unfounded. In our study, there was no aggression between dogs moved into pair housing. To reduce risk of aggression from larger groups, pair housing is recommended as a reasonable compromise between additional opportunities for social interaction versus for conflict. Hubrecht (1993) reported that dogs in pairs spend a similar proportion of their time interacting with each other as dogs kept in groups of 5–11 animals. However, it is important to choose potential living pairs carefully to reduce risks of aggression and stress for both dogs and staff (Wells 2004).

Unlike in some facilities where research on housing conditions has been conducted, dogs in our facility did have regular exposure (ranging from 30 to 120 minutes per day) to human handlers and other dogs, both in student teaching labs and through the facility's enrichment programme. This previous exposure may have contributed to the dogs' compatibility when in pair housing, but also may have dampened the differences between the two groups of dogs, once the experimental dogs were moved into pair housing. Supporting this possibility is the observation that the two dogs that did

not increase their socialising time when paired (pair 3) had experienced regular socialising with each other for many months prior to the study. One of these dogs was considered highly anxious ('Clove'; Paired 3B), and its companion ('Georgia'; Paired 3A) proved to have a calming effect. These two dogs were often together for teaching labs, etc, making the study treatment less novel for them. As well, 'Clove' was on anxiolytic medication prior to and throughout the study period (table 1), possibly minimising behavioural changes in solitary versus pair housing. Finally, cohoused dogs were separated at night, using the guillotine-style door between their two adjacent kennels. It is feasible that this reduction in time spent cohoused during each 24-hour period may also have dampened any effects of shared housing on these dogs' behaviour.

In the RUSVM kennels, we did not see self-mutilation, a behaviour commonly associated with dogs under chronic stress, but we did see stereotypical, repetitive behaviours such as spinning and jumping. Stereotypic behaviours, hypothesised to reduce arousal and HPA activity (Koolhaas and others 1999), represented only a minor component of the behavioural budget of our dogs (table 2).

Given how variable serum and salivary cortisol is known to be, with faecal cortisol being only slightly less so, stress-related glucocorticoid hormones in hair (which reflect weeks to months of stress perceived by individuals) are a more meaningful reflection and indicator of wellbeing. We recorded very high levels of glucocorticoid responsive compounds in hair from all the dogs, both preintervention and postintervention, compared with cortisol levels reported in other canine studies using similar methods: 11.6±0.8 pg/mg (Bryan and others 2013), 10.9±0.6 pg/mg (Bennett and Hayssen 2010) and 9.8±11.4 pg/mg (Piva and others 2008). These samples were analysed in the same lab, by the same people, using the same Salimetrics assay kit, as all previous and subsequent analyses. Because of the unusual results, several samples, including a pooled hair sample plus the standards, were run on every plate, but produced the same results. There is no obvious explanation for these remarkably high levels compared with previous studies, other than to discuss the potential flaws inherent in any ELISA-based assay. Since cortisol-related metabolites have not been well studied in dog hair, it is not yet possible to test cross-reactivity using the ELISA kits. Therefore, we must consider the likelihood that other, non-cortisol, glucocorticoid compounds were cross-reacting with the capture antigens on the ELISA plate. For this reason it is more correct to refer to the ELISA results as 'glucocorticoid responsive compounds' rather than cortisol, although we normally expect and assume the predominant steroid is cortisol. Whatever the explanation, the relative amounts were comparable among the study dogs, so they apparently all had the same 'cross-reacting' compounds.

Cortisol levels decreased significantly in all dogs from the baseline through the treatment period. Although the decrease tended to be greater for the pair-housed than the solitary dogs, the solitary dogs that were nearby may have experienced spillover positive effects (eg, due to reduced levels of barking during the treatment period). As with the behavioural data, the small sample size made statistical significance elusive for proving reduced stress in these dogs. We had a mix of males and females, intact and neutered dogs, but hair cortisol is not affected by age or neuter status (Bennett and Hayssen 2010). All our dogs were mixed-breed 'island dogs', so breed could not influence our results. The first (baseline) sample reflected a cumulative average of cortisol from many months of stress, whereas the second (postintervention) sample reflected only eight weeks of growth. This could complicate interpretation if stress was experienced differently over the growth of the hair, but all the dogs in our study had been there for at least six months prior to the housing changes. In addition, all study dogs had short hair coats with minimal 'under coat'. This coat type is continuous shedding as is common given the lack of seasonality in St Kitts. There were also no obvious physiological stressors such as recent whelping. We are confident that the baseline hair cortisol levels reflected a similar duration of cortisol deposition for all the dogs.

Hennessy (2013) notes that the HPA axis, the body's primary stress response system, appears particularly sensitive to stressors faced by dogs living in shelter conditions, similar to those experienced by dogs in the RUSVM kennels. However, the longer the exposure to stressors, the more difficult cortisol measures are to interpret (Hennessy 2013). In some reports, the natural negative feedback loops in the HPA system may result in downregulation and reduced, circulating glucocorticoids under prolonged exposure to stressors (Yehuda 2001, cited in Hennessy 2013). However, in studies of chronically stressed rats, adrenal hypertrophy and hyperplasia result in continuing high levels of circulating corticosterone (Ulrich-Lai and others 2006, cited in Hennessy 2013). Similarly, Beerda and others (2000) reported that dogs in markedly suboptimal housing conditions had elevated urinary cortisol levels that persisted for years.

In conclusion, in this pilot study we present preliminary behavioural and hormonal support for the hypothesis that pair housing is beneficial in reducing stress in kennelled dogs, consistent with previous studies of this topic. The behaviours most responsive to the housing change were active vigilance and repetitive movements (jumping and pacing), with some evidence that barking may prove a good measure as well. The long-term stress as reflected in hormone deposition in hair also provided support for the hypothesis that the dogs experienced lower stress levels when in paired housing. We interpret the decline in cortisol levels to be supportive of the beneficial nature of the housing change to this teaching dog colony as a whole. Based on our pilot study, our recommendations for future studies are as follows:

- Active vigilance and repetitive movements (such as jumping and pacing) should be used to

indicate changes in stress levels, with barking as an additional possibility for a behaviour representative of background stress levels.

- Larger sample sizes (while not feasible for our study) are necessary to compensate for the wide natural individual variation between dogs. In addition, we recommend controlling as much as possible other potential sources of variation such as age, sex, neuter status, previous experience and temperament.
- Measuring immunoreactive cortisol in hair provides an interesting new tool that will help investigators better understand the effects of their interventions on chronic stress in dogs. Aspects of this novel methodology will benefit from further validation, which can build on the baseline information in this, and other published work.
- In terms of understanding canine responses to chronic stress over time, and how these are reflected in hair cortisol analysis, it would perhaps be informative to use parallel methods of cortisol measure (eg, hair cortisol, in conjunction with repeated faecal cortisol sampling throughout the study period) following a housing change or similar stressor. Information gained from these two approaches (from the same dogs, during the same time period) could then be compared. Note that Mack and Fokidis (2017) did a similar comparison for cortisol measured from canine nail and hair samples, and found a significant positive correlation between the two methods.

Anecdotally, staff, faculty and students at RUSVM who interacted with the dogs communicated overwhelmingly positive feedback about the housing change. Domestic dogs are social animals, and numerous indications of potential benefit were recorded with no negative impacts seen, and thus we recommend pair or group housing of compatible dogs as a measure to increase welfare of kennelled dogs.

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Emma K Grigg, Belle Marie Nibblett, James Q Robinson and Judit E Smits

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