

Faecal corticosterone levels of dogs relinquished to a shelter in Japan

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ABSTRACT

This study investigated the baseline level of faecal corticosterone in dogs relinquished to a shelter and compared changes in it caused by social events that dogs experienced. Faecal corticosterone was measured to assess the average stress levels of dogs that depend on the actual conditions of the shelter. One category of subject animals was dogs relinquished by their owner or stray dogs who were penned in a group (group RG) or caged individually due to their incompatibility (group RI), and the other category was demonstration dogs kept for dog training classes (group DT) and petting activities held in welfare facilities for the elderly and in elementary schools (group DP). While the number of dogs that were included in groups RG and RI fluctuated almost daily, the demonstration dogs were kept together for three months. The average level of faecal corticosterone was significantly higher in group RG than in groups DT and DP. The level of group RI was also relatively high, but the difference from the other three groups was not statistically significant. Faecal corticosterone concentrations of groups DT and DP rose somewhat from the baseline levels on the day after social service activities. The results of this study reaffirm that temporarily relinquished and socially unstable dogs especially suffer a certain level of stress in a shelter.

INTRODUCTION

An estimated 70,000 dogs are relinquished to shelters yearly in Japan, and approximately 50 per cent of them are euthanased ([Nature Conservation Bureau of the Ministry of the Environment, Government of Japan 2012](#)). However, >30,000 relinquished dogs are readopted and their lives saved. Thus, public shelters play an important role in dog rehousing. The Kanagawa Animal Protection Center (KAPC) is a typical public shelter in Japan that takes in >600 dogs a year in Kanagawa Prefecture and succeeds in rehousing >80 per cent, one of the highest rates in all 47 prefectures in Japan ([Kanagawa Animal Protection Center 2013](#)).

Many shelters, including the KAPC, also conduct several public animal-related projects such as educational programmes for owners, dog training classes and petting activities ([Kanagawa Animal Protection](#)

[Center 2013](#)). Demonstration dogs used in these projects are selected from the relinquished dogs based on their characteristics as assessed by the staff of the KAPC. The petting activities are regularly held at local welfare facilities for the elderly and at elementary schools as well.

While being accommodated at shelters saves the lives of many relinquished dogs, dogs in a shelter suffer acute and/or chronic stress ([Stephen and Ledger 2006](#)). Introduction to novel facilities causes initial and prolonged stress to dogs ([Rooney and others 2007](#)). Also, it has been demonstrated that shelter dogs show more fear-appeasement behaviour towards an unfamiliar human ([Barrera and others 2010](#)). For sensitive dogs, living together and having social interactions with other dogs in the same room can be a potential stressor ([Carrier and others 2013](#)).

Stressors can induce adrenocorticotropic hormone (ACTH) release, which, in turn, increases the synthesis and secretion of cortisol by the adrenal cortex. As a result, blood cortisol concentrations have been widely used as an indicator of stress. However, blood sampling is itself invasive and stressful for animals. Therefore, a non-invasive approach for monitoring physiological responses to stress has been sought. Lately, it has been confirmed that measuring cortisol metabolites in faeces can be a useful non-invasive tool for monitoring stress in carnivores ([Monfort and others 1998](#); [Schatz and others 2001](#)).

The aim of this study was to gather information about the stress level of dogs relinquished to a shelter. The authors also measured the stress level of dogs kept in the shelter after they experienced social events. They also hypothesised that training classes and petting service activities may be stressful for dogs. Faecal corticosteroid was measured to assess the stress level of dogs as a non-invasive method for evaluating adrenal activity ([Monfort and others 1998](#); [Schatz and Palme 2001](#)).



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MATERIALS AND METHODS

Animals and housing

Dogs housed at the KAPC were recruited as subjects. They were broadly classified into two main groups based on the reasons they were kept: one was dogs relinquished by the owner or stray dogs (group R), and the other was demonstration dogs kept for social activities (group D). Dogs in group D were selected from dogs in group R (as described above) and were engaged in social activities for one year on average. Group D was further divided into two subgroups: one was dogs used for dog training classes (group DT: three females and three males), and the other was dogs used for petting activities held at local welfare facilities for the elderly and at elementary schools (group DP: two females and two males). Dogs in group R were never used for these social activities. Group R occasionally included dachshund, Maltese, Shih Tzu, Shiba-inu, labrador retriever and Akita-inu breeds, but most relinquished dogs were mongrels. The ages of most dogs were unknown.

Groups DT and DP were kennelled individually outdoors (group DT: 0.9×1.0×1.85 m/kennel; group DP: 3.0×3.9×1.95 m/kennel). Group DT was taken out for a walk for about 30 minutes in the morning. They were trained to follow basic commands including 'sit', 'wait', 'come', 'down' and 'heel' by specific staff members for 15 minutes after the walk, and then allowed to access the outdoor play area until 14.00. Positive reinforcement-based training was used at the daily training as well as in the training class. After 14.00, they were kennelled. Group DP was pressed into service on Tuesday, Wednesday and Thursday. The activity took two or three hours each time including travel by car. The dogs in groups DT and DP were kept with the same group members for a relatively long period of time (averaging several months). Afterwards, they were put up for adoption.

Group R was subclassified into group-housed (group RG: range of 1–8 dogs/pen) and those caged individually (group RI: small cages: 2–5 dogs; large cages: 3–8 dogs). Group RG consisted of 6 pens (4.5×5.5×3.3 m/pen) and group RI consisted of 13 cages (5 small cages: 0.8×1.5×0.7 m/cage and 8 large cages: 1.0×2.0×2.5 m/cage). Groups RG and RI were kept indoors all day without outdoor exercise or positive human contact such as a walk. The number of dogs accommodated in groups RG (range of 16–24 dogs) and RI (range of 6–13 dogs) fluctuated almost every day. All dogs were fed twice a day and allowed free access to water.

Faecal sample processing and enzyme immunoassay

Faeces were collected daily from individuals in groups DT, DP and RI for two months from February to April during the morning routine. In group RG, more than one sample was collected from each pen, and the averaged data were used for statistical analysis as described below. Only faeces that were normal and fresh at the time of sampling were collected. Watery faeces were not

gathered. Individual faecal samples were stored in a polypropylene bag and immediately frozen at -80°C until drying. The faecal samples were dried in a drying oven (SDN27; Sansyo, Tokyo, Japan) at 100°C for >16 hours and then powdered with a mill (IFM-800DG; Iwatani, Osaka, Japan). Powdered faeces were extracted using a vortexing method modified from [Wasser and others \(2000\)](#). Briefly, 0.1 g of dried sample was placed in a 2 ml microtube with 1 ml of 95.5 per cent ethanol, capped, vortexed (5 minutes) using the vortex mixer (Vortex-Genie2; MandS Instruments, Osaka, Japan), and then centrifuged (CN-820; AS ONE, Osaka, Japan) for 5 minutes at 1050 g. The faecal corticosterone concentration of the supernatant was determined using an enzyme immunoassay kit (ADV900-097; Cosmo Bio, Tokyo, Japan). The extraction efficiency of exogenous corticosterone using this method was 65.1 per cent. All faecal concentration data are expressed as nanograms per gram of dry faeces.

Statistical analyses

Data were analysed using the statistical software program Statcel3 (V.3, 2011; OMS Publishing, Tokyo). New dogs were introduced to the pens or cages and/or remixing of penmates occurred almost every day so that it was not possible to determine baseline concentrations for groups RI and RG. The number of dogs included in each group changed from day to day, so the mean corticosterone concentration of each group on each sampling day was used for analyses. The numbers of sampling days used for analysis were 19 and 20 days in groups R and D, respectively.

Next, the authors analysed data of the baseline concentration and response to potentially stressful events of each group in more detail. It is reported that the faecal corticosterone concentration reaches the peak at 24 hours after stressful events such as ACTH administration ([Monfort and others 1998](#)). Therefore, the mean value of faecal corticosterone concentrations on the days after the dogs in each group did not perform potentially stressful events was used as the baseline concentration. For a comparison between days before and after dogs engaged in each social service, six and four repetitions of measurements were carried out during the collection period for groups DT and DP, respectively.

Mann-Whitney U tests and Kruskal-Wallis analysis of variance followed by Steel-Dwass test for multiple comparisons were used to compare groups. For significance, a P value of <0.05 was selected. Results are reported as mean±sd.

RESULTS

[Figure 1](#) shows the patterns of the variation per day of the mean faecal corticosterone concentrations in each group during the collection period. The numbers of sampling days used for analysis were 19 and 20 days in groups R and D, respectively. Mean faecal corticosterone concentrations fluctuated from day to day, particularly in

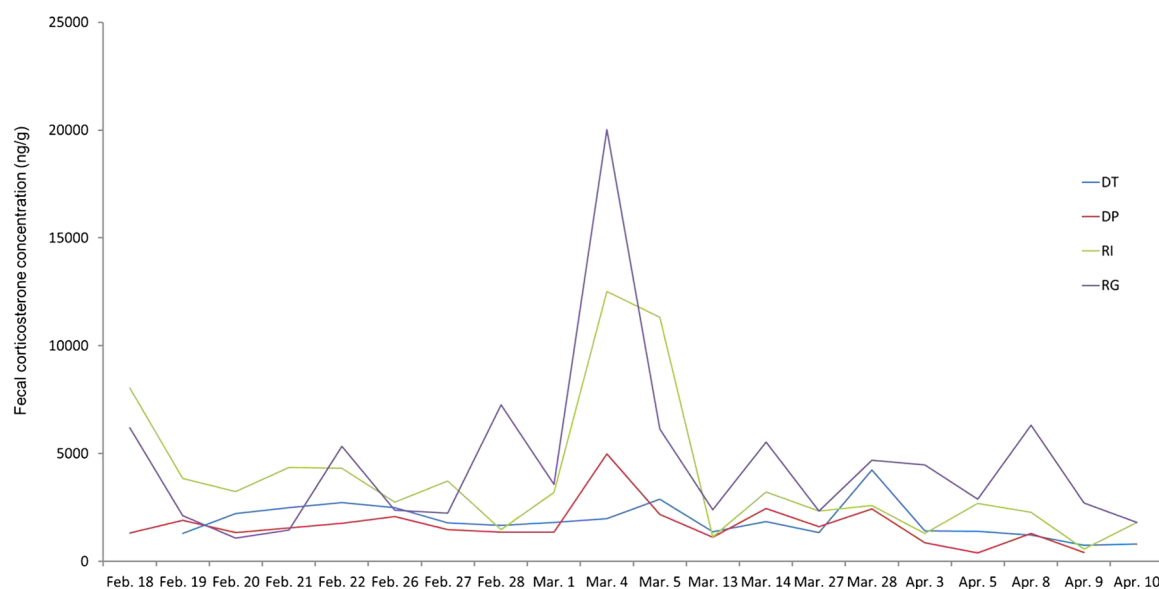


FIG 1: Daily variation in faecal corticosterone concentration expressed as mean values for each group. DT, demonstration dogs kept for dog training classes; DP, demonstration dogs kept for petting activities at local welfare facilities for the elderly and at elementary schools; RI, dogs relinquished by their owner or stray dogs who were caged individually; RG, dogs relinquished by their owner or stray dogs who were penned in a group

groups RG and RI, and there was a significant difference among groups ($H=20.51$, $df=3$, $P=0.0001$). The overall mean faecal corticosterone concentration was significantly higher in group RG (4549.3 ± 4046.4 ng/g, $n=19$) than in groups DT (1884.7 ± 830.8 ng/g, $n=20$, $P<0.01$) and DP (1679.3 ± 985.2 ng/g, $n=20$, $P<0.01$). The mean concentration of group RI (3836.9 ± 3139.7 ng/g, $n=19$) was relatively high, but it was not statistically different from those of the other three groups (Fig 2).

Figure 3 shows differences in the baseline and mean faecal corticosterone concentrations on the days after dogs engaged in social service. Baseline concentrations of faecal corticosterone were not statistically different between groups (group DT: 1035.9 ± 440.7 ng/g, $n=6$;

group DP: 1019.1 ± 619.4 ng/g, $n=4$, $P=0.4624$). In group DT, the faecal corticosterone concentration rose significantly to 3596.3 ± 1286.0 ng/g from the baseline concentration on the day after training ($U=36$, $P=0.0039$). In group DP, the faecal corticosterone concentration also significantly rose to 2891.3 ± 1606.6 ng/g ($U=16$, $P=0.0209$). Faecal corticosterone concentrations on the day after training were not statistically different between groups ($P=0.3938$).

DISCUSSION

The first aim of this study was to gather information about the stress level of dogs relinquished to a shelter.

FIG 2: Faecal corticosterone concentrations expressed as mean and sd for each group showing the results of analysis using Steel-Dwass non-parametric equivalent test. DT, demonstration dogs kept for dog training classes; DP, demonstration dogs kept for petting activities at local welfare facilities for the elderly and at elementary schools; RI, dogs relinquished by their owner or stray dogs who were caged individually; RG, dogs relinquished by their owner or stray dogs who were penned in a group

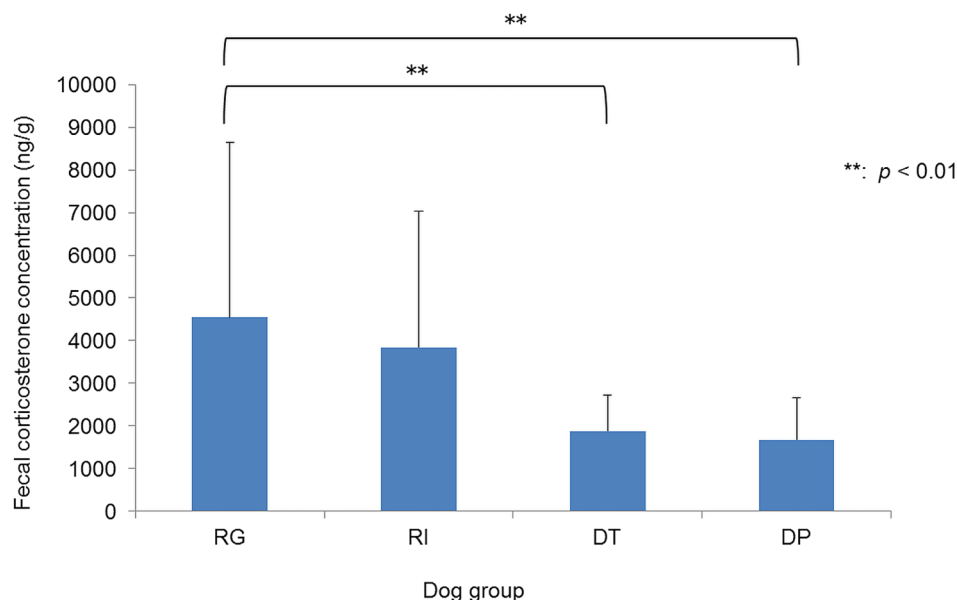
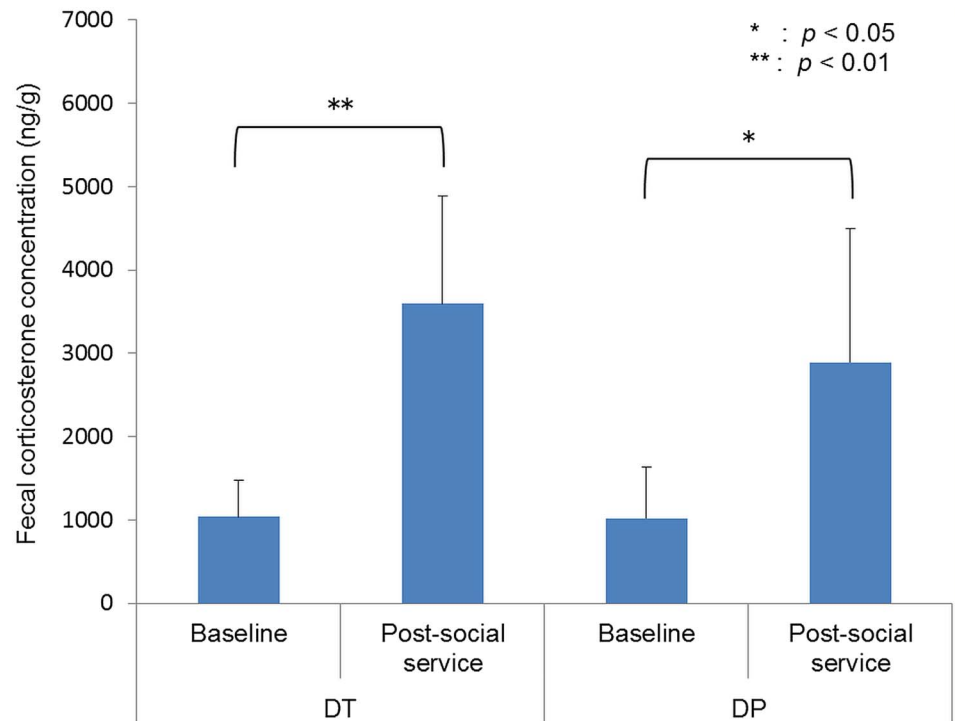


FIG 3: Faecal corticosterone concentrations expressed as mean and sd for each group at baseline and on the day after each social service. Significant differences from the results of Mann-Whitney U test are shown. DT, demonstration dogs engaged in dog training classes; DP, demonstration dogs engaged in petting activities at local welfare facilities for the elderly and at elementary schools



Hundreds of dogs are yearly relinquished to the KAPC for various reasons, so the species and histories of the dogs before they entered the shelter were varied (Kanagawa Animal Protection Center 2013). Thus, these factors might lead to the large variation in the raw data obtained in this study. Nevertheless, the results of this study reaffirm that dogs relinquished to a shelter (group R in this study) suffer a certain level of stress (Stephen and Ledger 2006). The average level of their faecal corticosterone was at the same level as the faecal corticoid excretion after an exogenous ACTH (400 IU) challenge (Monfort and others 1998). Previous studies have demonstrated that introduction to novel facilities (Rooney and others 2007) and mixing with unfamiliar dogs (Carrier and others 2013) can cause initial and prolonged stress to dogs. In addition to this, it is reported that shelter dogs show a more fear-appeasement behaviour towards an unfamiliar human (Barrera and others 2010). Furthermore, the lack of positive human contact by group R in this study might cause considerable stress. There is evidence for the benefits of positive human contact on stress responses in some situations (Taylor and Mills 2007). In addition to these external factors, dogs' temperaments (De Palma and others 2005) and their history, that is, whether the dogs were strays, relinquished from homes or returned to the shelter for a second time (Hiby and others 2006), may have affected their sensitivity to stress, although the authors analysed the data without extracting these factors.

Second, the authors hypothesised that training classes and petting service activities may be stressful for dogs. However, the results of this study indicated that

demonstration dogs (group D in this study) might originally have had higher stress tolerance since they were selected for their temperaments (De Palma and others 2005). Furthermore, they had enough time to become accustomed to the shelter environment. Even so, it was suggested that even demonstration dogs that are kept in relatively comfortable conditions in a shelter may feel some stress (Monfort and others 1998) when they engage in the social services of dog training classes and petting activities. It has been shown that irregular exercise schedules are associated with higher cortisol concentrations in military working dogs and that this elevation lasts for several weeks (Lefebvre and others 2009). Thus, the stress that such dogs experience would be reduced if social activities were scheduled regularly and conducted on fixed days every week. In relation to this point, it is reported that experience is the prime factor in reducing stress in avalanche rescue dogs during search missions (Slotta-Bachmayr and Schwarzenberger 2007). The authors also reported that the mean elevation of the noradrenalin concentration decreases linearly as days of participation in animal-assisted activities pass, even in initially inexperienced dogs (Uetake and others 2007). Therefore, consideration by staff members of differences between dogs in manner of coping with social stress (Horváth and others 2007) would reduce the stress load during activities as well.

This study was a case report in an animal shelter in Japan, so further investigations will be necessary for generalisation of the results obtained here. However, it seems that human intervention through training and petting activities reduces the baseline stress level of dogs kept in animal shelters.

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Contributors KU designed this study. TT gave advice about an experimental design as a senior researcher. HU, JI and SK carried out this study at their animal shelter. All authors discussed the results of this study.

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Competing interests None declared.

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