

Marking frequency during intraspecific socialization sessions is related to urinary cortisol levels in shelter dogs

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Summary

The aim of this study was to determine whether behavioural indicators such as marking frequency and whether cortisol/creatinine ratio (C/Cr) are influenced by three socialization sessions. Six adult shelter dogs were selected: Group 1 (n.3) with adequate social experience and Group 2 with behavioural problems of aggression against humans (n.2) and excessive fear response towards conspecifics (n.1). Spontaneous urine samples were collected for C/Cr after socialization sessions. C/Cr was positively correlated with urinary marking frequency ($P<0.01$) and negatively with faecal marking frequency ($P<0.05$). The correlation with urinary marking could be related to increased release of aldosterone under ACTH stimulus or to chemical information that dogs are in a stressful situation. The negative correlation with frequency of faecal marking could be related to inhibition of act in a novel situation as stress effect. Non-invasive monitoring for detection of cortisol could be useful to assess canine behaviour modulation following intraspecific socialization sessions.

Keywords: urinary cortisol; shelter dog; marking

Introduction

Examination of social behaviour is very important for screening shelter dogs to be placed for adoption or for identifying dogs that need behavioural intervention. Shelter should provide behavioural rehabilitation for fearful and un-socialized dogs in order to increase their chances of adoption. Intraspecific socialization should be an important component of behavioural rehabilitation of un-socialized dogs. Intraspecific social exposure sessions could be useful to modulate undesirable behaviour in owned dogs (Distefano and Alberghina, 2016) but also to increase social behaviour and working ability in military dogs (Gfrerer et al., 2018). Urinary cortisol:creatinine ratio (C/Cr) is probably the most widely used physiological indicator, which is reported in published studies about canine welfare (Hewson et al., 2007) but individual variability and lack of specificity as a stress response have led researchers to question the value of glucocorticoid levels as a welfare indicator (Part et al., 2014). In this study, we hypothesized that social exposure sessions could affect cortisol and behaviours, such as urinary and faecal marking, in shelter dogs.

Material and Methods

Six adult gonactemized dogs were chosen from the animal shelter in Rovereto (TN), Italy. They were all housed in the shelter for six months or more. Group 1 (Wallace, Acab and Frida) with adequate social experience and Group 2 with behavioural problems of aggression against humans (Asia and Cloe) and excessive fear response towards conspecifics (Baloo)(Table 1). Each dog of Group 2 had the opportunity to have contact with one conspecific of Group 1 within the fenced area (10 m×20m) from 12.00 to 12.30 pm. Frequency of urination and defecation was monitored.

Table 1. Characteristics of gonactemized dogs used in the study

Dog	Estimated age (Years)	Gender	Breed	Group
Wallace	7	M	German Sheperd	I
Acab	3	F	German Sheperd	
Frida	7	F	Crossbred	
Baloo	7	M	Hound	II
Cloe	2	F	Crossbred	
Asia	9	F	Argentin Mastiff	

In a first phase, the experimenter controlled the dog with a leash while direct contact with conspecifics was allowed. In a first phase of social sessions, all dogs wore a baskerville muzzle to ensure that no dog could get harmed. The dog trainer supervised all training and decided which dog wore a muzzle or when a dog could be off the leash. The behaviour of the dogs during social exposure session was video recorded. Spontaneously voided urine samples were collected 6 hours after the end of socialization session when subjects were let out of their box. Urine was collected by P-scoop dog urine collector and transferred in sterile cups. Samples were frozen at -20°C until further analysis for cortisol and creatinine. Cortisol in urine was analysed by means of a microtiter plate reader (EZ Read 400 120 ELISA, Biochrom, Cambridge, United Kingdom) with a commercial cortisol urine-ELISA kit according to the manufacturer's instructions (Cortisol Urine ELISA, LDN GmbH & Co. KG). Urinary creatinine (Cr) was also assessed in order to index, and control for, varying urine concentration levels. Creatinine assessment was completed with a commercially available kit using a modified Jaffe method (GIESSE Diagnostics srl, Italy) by means of a UV spectrophotometer (model Slim SEAC, Firenze, Italy). Urinary cortisol, corrected for creatinine, to give urinary cortisol creatinine ratio (C/Cr nmol/ μmol) was calculated. Analysis of variance (ANOVA) with repeated measures was used to assess the influence of sessions on C/Cr. Bonferroni's multiple comparison test was applied for post hoc comparisons. Pearson's linear correlation was used to evaluate the relationship between urinary and faecal frequency marking and C/Cr. All the statistical analyses were performed using Statistica 8 software (Statsoft Inc., Tulsa, OK, USA). P-values <0.05 were considered statistically significant.

Results

Levels of C/Cr after sessions were significantly influenced by time ($F=6.196$; $P<0.05$, Fig. 1). A significant positive correlation was found between C/Cr and urinary marking frequency ($r=0.654$, $P<0.01$ Fig.2). A negative correlation was found between C/Cr and faecal marking frequency ($r=-0.546$ $P<0.05$, Fig. 3).

Figure 1. Influence of social sessions on C/Cr levels in six shelter dogs

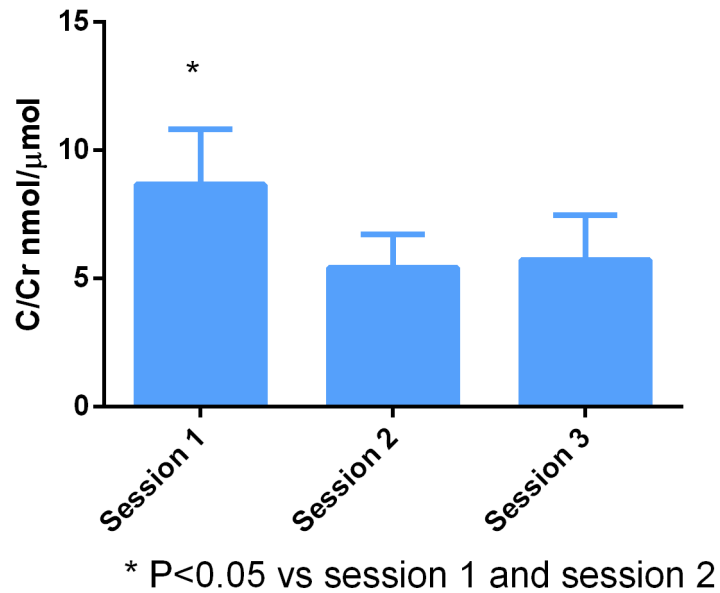


Figure 2. Linear correlation between C/Cr and urinary marking frequency

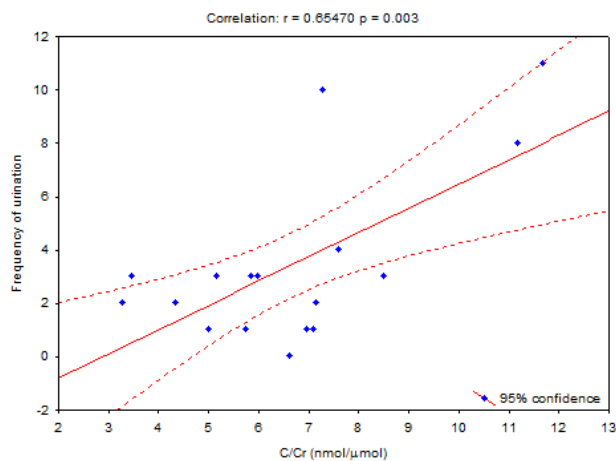
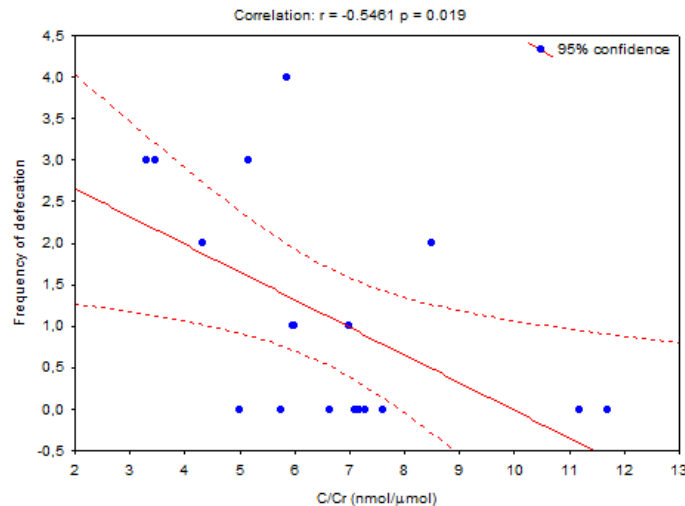


Figure 3. Linear correlation between C/Cr and fecal marking frequency



Discussion

Urine capture is painless, non-invasive, and inexpensive, which is an advantage over other measures gained by invasive techniques (e.g. from serum or plasma). Cortisol levels in urine reflect a stress response in the previous 4-8 hours (Casey, 2005). Levels of cortisol, measure the metabolic rate of the animal and increase during arousal in positive events as well as negative events, therefore, it is possible that the elevated cortisol levels after social exposure were due to arousal and the resulting increase in activity and not necessarily an indication of stress (Protopopova, 2016). The small group of socialized dogs showed a tendency to have lower cortisol levels, lower frequency of urination and higher frequency of defecation than the small group of dogs with behavioural problems. The positive correlation between C/Cr and frequency of urinary marking could be related to the increased release of aldosterone under ACTH stimulus or to chemical information that dogs are in a stressful situation. No previous research on canine marking behaviour has evaluated relationship with urine cortisol levels. Previously, in relation to acute stress, no significant correlations between behavioural and physiological parameters (salivary cortisol and heart rate) have been observed (Beerda et al., 1998). Tod et al. (2005) reported that defecation and urination were excluded from analysis of a behavioural test for

efficiency of dog appeasing pheromone, in relation to stress and fear related behaviour due the absence or rarity of their occurrence during testing.

The negative correlation between fecal marking frequency and C/Cr levels in this study, could indicate that in a novel situation related to intraspecific social exposure, defecation could be inhibited. Further studies are necessary to evaluate the role of marking communication between dogs in relation to arousal and stress.

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