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Research

Salivary cortisol concentration in healthy dogs is affected by size, sex, and housing context

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ABSTRACT

The aim of the article was to investigate the effect of site of sampling, size, and sex on the variations of salivary cortisol of healthy dogs. Samples of saliva were collected from dogs of private owners (n = 13), kennels (n = 4), and shelters (n = 2). For each dog, samples were collected at the first interaction of the day with man (T0) before the morning meal (6:00-8:00 AM), 30 minutes after the meal (T1), and 30 minutes after the last interaction of the day with man (T2), when dogs were resting and apparently relaxed. A total of 92 dogs belonging to 17 different pure breeds or crossbred were eligible for the study, being 19 dogs privately owned, 47 recruited in kennels, and 26 hosted in shelters. Salivary cortisol concentrations of the dog population were not normally distributed, and data were transformed to natural logarithm (ln). The mean values ranged from −0.70 to 3.40 ln ng/mL, with an average of 0.90 ± 0.76 ln ng/mL, corresponding to 0.50, 30.00, and 3.48 ± 4.05 ng/mL. Mean salivary cortisol was significantly higher for dogs hosted in shelters than those privately owned or in the kennels ($P < 0.05$). Cortisol values from intact dogs did not differ between males and females, whereas for castrated males and spayed females, significantly lower values were found ($P < 0.01$ intact vs. castrated males; $P < 0.05$ intact vs. spayed females). Mean salivary cortisol concentration was significantly lower for giant and large-sized dogs than for small-sized dogs ($P < 0.01$), whereas mean cortisol for medium-sized dogs was not significantly different from the other sizes. The interaction of site with time of sampling was significant ($P < 0.05$), with the highest cortisol concentration at T2 for dogs privately owned and housed in the kennels and at T0 for dogs hosted in the shelters. This study, focused on healthy dogs, indicated that several factors can affect the concentration of salivary cortisol. Further studies also involving pathological conditions are required to identify critical values that can be used for clinical management settings.

Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is exquisitely sensitive in the transduction to biological responses of the exposure to cognitive and noncognitive stress. Cortisol is the main end product of the activation of HPA axis and is widely used and accepted to monitor the reactivity to stress, also in connection to disease (Gallagher and Ritsner, 2009; Hellhammer et al., 2009).

Cortisol concentration can be measured in several matrixes, as blood, urines, feces, integument, milk, or saliva, each of them enabling the study of HPA axis from different perspectives (Bennet and Hayssen, 2010; Meyer and Novak, 2012). For chronic stress, hair has been often proposed because it accumulates a series of repeated and sometimes different stimuli in the period to which they refer, although color, climate, and other factors can affect at some extent the reliability of this matrix for long-term assessment (Bennet and Hayssen, 2010). In the case of an evaluation of the short-term response to stimuli, blood, tears, and saliva can be preferentially used, and the latter is the more accessible and easier to collect, minimizing animal restrain (Kobelt et al., 2003).

Study on salivary cortisol concentrations to mark distress or undesirable outcomes in dogs is a topic that has received an increased attention in the last years (Bellaio et al., 2009; Bennet and Hayssen, 2010; Wenger-Riggenbach et al., 2010; Beetz et al., 2011; Pastore et al., 2011; Glenk et al., 2014). Saliva can be collected less

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invasively than blood or urine, and its cortisol concentration has shown to closely parallel plasma cortisol values (Beerda et al., 1996; Hellhammer et al., 2009). The vast majority of these researches used cortisol measurements to investigate stress-related response in dogs or to correlate HPA axis response to behavior, with the aim of using a biological marker to assess cognitive or noncognitive stress, also providing a practical tool for clinical management settings. However, these studies were more focused on salivary cortisol variations in relation to the specific experimental design than to assess physiological differences among genetic and environmental contexts as well as other animal-related factors, as size and sex.

The aim of the article was to investigate the variations of salivary cortisol concentrations during an ordinary day of the life in healthy dogs. Salivary samples were collected from dogs at home, kennels, or shelters.

Materials and methods

Animal selection

Dogs were recruited from 13 private owners, 4 kennels, and 2 shelters, all located in the North East part of Italy. The aims of the study and the sampling procedures were disclosed to the owners, breeders, or shelter managers, and informed consents were obtained before any procedure. After showing instructions and training, owners, breeders, or shelter personnel were the only people who collected saliva samples, to avoid potential interference because of the presence of unknown people.

The criteria for dogs to be eligible for the study were the following: (1) more than 12 months of age; (2) clinically healthy, free from pain, external and internal parasites, and immunized, as assessed by a veterinary practitioner; (3) without history of neurologic abnormalities; (4) without history of behavioral problems; (4) no recent history of corticosteroid administration; and (5) no drug therapy at sampling and from 1 month before. Clinical data were obtained from the medical records available from the owners or shelter and kennel veterinarians.

For each dog, the following information was also collected: date of birth, breed, size, sex (male, castrated male, female, and spayed female), feeding schedule, and type of food. For each dog, 3 samples were collected during the same day. The T0 sample was collected in the morning at the first interaction with man immediately before the morning meal (6:00-8:00 AM) and the T1 sample 30 minutes after the meal. The last sample (T2) was collected 30 minutes after the last interaction of the day with man, when dogs were resting and apparently relaxed, according to the visual evaluation of the personnel in charge of the animal.

Salivary sampling

To avoid contamination of samples and the interference with the enzyme immunoassay (Dreschel and Granger, 2009), dogs were to refrain from drinking and eating 20 minutes before sampling. Salivation was stimulated allowing the dogs only to sniff food treats (Bennet and Hayssen, 2010; Ligout et al., 2010). Saliva was collected with swabs (Salimetrics, State College, PA) gently placed into the cheek pouch of the dog by the owners, breeders, or shelter managers for approximately 90-120 seconds, a time considered adequate for the saturation with saliva. Samples were checked for visible contamination with food or blood. For ethical reasons, dogs were never restrained. After sampling, the swabs were introduced into tubes specifically designed to avoid cortisol sequestration (Salivette; no. 51.1534, Sarstedt, Nümbrecht, Germany), temporally stored in an iced box before the final storage at −20°C. Before analysis, performed within 15 days, swabs were thawed and centrifuged at room temperature at 1500×g for 15 minutes to obtain clear saliva, which was used for cortisol determination using an enzyme immunoassay kit (Salimetrics, State College, PA) (Hekman et al., 2012). Samples were assayed in duplicate, using 25 μL of sample per well. The kit's lower limit of sensitivity was 0.03 ng/mL. Average intra- and interassay coefficients of variation were less than 12% and 8%, respectively.

Statistical analysis

For the analysis, only dogs with all the 3 daily samples were considered, and a total of 92 dogs were available. Data were stored in a spreadsheet using Microsoft Office Excel (2010; Microsoft Corp, Redmond, WA), and the descriptive statistics and analyses were performed with the SPSS (1997) package (SPSS Inc, Chicago, IL). Normality of salivary cortisol was tested by the Kolmogorov–Smirnov nonparametric test. Because data were not normally distributed, natural logarithm (ln) transformation was adopted. Dogs were classified according to the Federation Cynologique International (http://www.fci.be/en/) in small, medium, large, and giant sizes. For statistical analysis, a mixed model was used for the natural logarithm-transformed values, considering the fixed effects of site of sampling (owner, kennel, and shelter, from 1 to 3), sex (males, castrated males, females, and spayed females, from 1 to 4), size (small, medium, large, and giant, from 1 to 4), time of sampling (T0, T1, and T2, from 1 to 3), the random factor of subject repeated with time of sampling, and the covariate of age within size. To identify differences between means, the least significant differences test was applied.

Results

A total of 92 dogs, belonging to 17 different pure breeds or crossbred, were eligible for the study. In particular, 19 dogs were privately owned, 47 were recruited in kennels, and 26 were hosted in shelters (Table 1). Most dogs were not neutered (26 males and 45 females), and the 7 castrated males came all from the shelters, whereas 8 of the 14 spayed females were from the shelters, 5 from private owners, and 1 from kennel. The most represented pure breeds within the population were dachshund, golden retriever, Irish wolfhound, and Jack Russell terrier (12, 11, 9, and 9 subjects, respectively). Seven pure breeds were represented by 1 dog each, and crossbred was the most numerous group (18 dogs). On average, dogs were 4.2 ± 3.4 years old.

The values of salivary cortisol were not normally distributed in our dog population, and a natural logarithm transformation was applied. After transformation, data were normally distributed (Table 2), showing lower values for symmetry indexes, skewness, and kurtosis. The mean salivary cortisol ranged from −0.70 to 3.40 ln (ng/mL), with a mean value of 0.90 ± 0.76 ln (ng/mL), corresponding to 0.50, 30.00, and 3.48 ± 4.05 ng/mL (Table 2).

The effects of size, sex, site, and time of sampling on natural logarithm-transformed cortisol concentrations are reported in Table 3. Cortisol concentrations significantly differed between sites of sampling ($P < 0.05$), having the highest values in dogs hosted in shelters, followed by dogs privately owned and by dogs in kennels (0.99, 0.58, and 0.46 ln [ng/mL], respectively). Cortisol values did not differ between intact males and female dogs, whereas for castrated males and spayed females, significantly lower values were found (0.21 and 0.47 ln [ng/mL], respectively; $P < 0.01$).

Mean salivary cortisol concentrations were significantly higher for small-sized dogs in comparison to the other sizes ($P < 0.01$). Giant and large-sized dogs had significantly lower mean cortisol concentration ($P < 0.01$) than small-sized dogs but did not differ