



A systematic review and meta-analysis of salivary cortisol measurement in domestic canines



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ABSTRACT

Salivary cortisol is widely used as an indicator of stress and welfare in canine research. However, much remains unclear about the basic features of this hormone marker in domestic dogs. This systematic review and meta-analysis aimed to determine a reference range for cortisol concentration in the saliva of dogs and examine how canine characteristics, environmental effects and experimental considerations relate to salivary cortisol concentrations. A systematic review of literature databases and conference proceedings from 1992 to 2012 identified 61 peer-reviewed studies using domestic dog salivary cortisol. Researchers were contacted via email, and 31 raw data sets representing a total of 5,153 samples from 1,205 dogs were shared. Meta-analysis provided a cortisol concentration range of 0 to 33.79 $\mu\text{g/dL}$ (mean 0.45 $\mu\text{g/dL}$, SEM 0.13). Significant effects ($P < 0.05$) were found for sex and neuter status, age, regular living environment, time in environment before testing, testing environment, owner presence during testing, and collection media. Significant effects were not found for dog breed, body weight, dog type, coat color, assay type, exercise, eating, or use of salivary stimulant. Care should be taken when using cortisol studies for dogs at a group or population level as there is a large amount of intraindividual and interindividual variability and external variables could influence salivary cortisol concentration. This analysis highlights the importance of carefully controlling experimental design to compare samples within and between individual dogs, as well as establishing and using best practices for saliva collection. Caution should be exercised in comparing different studies, as the results could be the reflection of a plethora of factors.

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1. Introduction

The relationship between stress, health, and well-being of domestic dogs is a significant area of scientific interest concerning canines in both companion and working contexts [1,2]. Although widely used as an indicator of stress and welfare in canine research, much remains unclear about the basic features of salivary cortisol in domestic

dogs. This systematic review and meta-analysis aimed to determine a reference range for salivary cortisol concentration in domestic dogs and to examine how canine characteristics, environmental effects, and experimental design may impact cortisol concentration in saliva.

1.1. Use of salivary cortisol concentration in domestic canine research

Cortisol is extensively used as a measure of hypothalamic-pituitary-adrenal axis (HPA) activity and is found in plasma, saliva, feces, urine, and hair of many

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species. In healthy dogs, cortisol concentration in saliva is highly correlated with plasma cortisol, as unbound cortisol passively diffuses from blood into saliva [3,4]. Illness and medications influencing protein binding may reduce the correlation between plasma and salivary cortisol concentrations. Saliva collection is relatively noninvasive and can be collected and stored at convenient and meaningful times of the day in a variety of settings. Although animal handling is required, saliva collection is tolerated well by most dogs and is not technically challenging, allowing people to be easily trained to collect samples [5]. Collection involves saturating absorbent collection material with saliva in the dog's mouth, with or without the presence of a salivary stimulant. It has been shown that if the collection procedure takes <4 min, there is no handling effect on the cortisol concentration of that sample [6]. Saliva is then extracted from the absorbent material, frozen for an interim period, and tested for cortisol concentration using validated radio- or enzyme-linked immunoassays.

1.2. Rationale for meta-analysis

Canine salivary cortisol concentration is used as a measure of animal welfare, generalized stress response, response to acute stimuli, and response to interventions in a variety of testing environments, as an indicator of aging and of diseases, such as hyperadrenocorticism. Intraindividual and interindividual variation in canine cortisol concentrations are often cited as limitations in salivary cortisol interpretation. With the increasing use of salivary cortisol concentration as a measure of canine stress and welfare in the literature, it is important to establish range limits and identify any canine, environmental and experimental effects on this popular marker. Meta-analysis is a formal, quantitative statistical technique for combining the results from multiple independent studies to systematically derive conclusions about that body of research. It allows results from studies that have great variability (study heterogeneity) to be pooled and analyzed effectively. We determined to use a random effects meta-analytic model that accounts for study heterogeneity. This provides confidence that the higher the study heterogeneity, then (1) the larger the resultant variance, (2) the wider the resultant CI, and (3) the smaller the chance of detecting statistical significance. This epidemiologic study design is widely used in medical research [7].

1.3. Objectives

1.3.1. Individual canine characteristics

The first objective of this study is to establish normal values and ranges for canine salivary cortisol concentration and to investigate the effects of age, sex, color, size, and neutering status on cortisol concentration. In addition, we seek to examine the effect of breed and purpose of dog, separately from environmental differences, on salivary cortisol concentration.

1.3.2. Environmental effects

Objectives of the study also include studying the effects of a dog's living situation on salivary cortisol concentrations. Previous research suggests that dogs living socially

with other dogs may not have as pronounced a cortisol response to a specific stressor as those that live alone [8]. Research also indicates that cortisol concentrations may vary between dogs sampled in their home environment; at a boarding kennel, rescue shelter, or veterinary hospital; in competition; or in a working or training kennel facility [9]. In addition, the dogs' familiarity with the testing environment, the duration they have been housed there, and whether their regular owner or handler is present at the time of testing could affect cortisol concentrations [10,11].

1.3.3. Experimental considerations

In many species, cortisol has strong diurnal, circadian, and seasonal rhythms of secretion. Although a number of studies have failed to show a diurnal rhythm in plasma or salivary cortisol concentration in dogs [12–15], Beerda et al [16] recorded significantly higher mean salivary cortisol concentrations in their canine subjects in the morning than during the rest of the day. This study seeks to investigate the role of sampling time on canine salivary cortisol concentrations further, with the capacity to compare many dogs from multiple studies and countries, across various time points.

Exercise has been shown to increase plasma cortisol concentrations in dogs [17], but the effect of exercise on salivary cortisol in dogs is unknown. Therefore, exercise may potentially pose a confounding variable in studies that include exercise as part of the experimental protocol [18,19]. In addition, although ingestion of a protein rich meal has been shown to increase salivary cortisol concentration for up to 2 h in humans [20], this response has not been tested in dogs to our knowledge.

Cortisol concentration has been measured in saliva using both RIA and ELISA. In addition, different saliva collection materials, as well as stimulants to increase salivary flow, have been used by canine researchers. Collection materials can affect determination of some salivary biomarkers and sample collection volume [5]. A final objective of this meta-analysis is to clarify any effects that differences in experimental methodology and study design might have on cortisol concentration results.

2. Methods

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses Statement guidelines to plan, implement, and report this systematic review and meta-analysis [21,22].

2.1. Identification of studies

We searched databases PubMed, Web of Science, Biological Abstracts (via Ovid), Scopus, PsychInfo, ProQuest, and Google Scholar for peer-reviewed studies using domestic canine salivary cortisol from 1992 to 2012. The keyword terms saliva*, and cortisol were used in addition to dog*, pup* or canine*. The proceedings for the Canine Science Forum 2008, 2010, and 2012 were visually scanned and electronically searched for cortisol, stress, and salivary. The International Society for Applied Ethology proceedings

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