



The influence of the duration of the preoperative time spent in the veterinary clinic without the owner on the psychogenic and oxidative stress in dogs

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ABSTRACT. The objective of this study was to evaluate the influence of the dog's long-term separation from its owner in the novel environment on the occurrence of psychogenic and oxidative stress. Group I dogs (n=9) were brought to the veterinary clinic and stayed in a kennel room for 12 hr before the surgery, and group II dogs (n=9) –for 10 min before the surgery. Physiological parameters (heart rate (HR) (beats/min) and respiratory rate (f_R) (breaths/min)) were measured and blood sampling was done 12 hr before the surgery (T0) for group I dogs and 10 min before the surgery (T1) for both groups dogs. Oxidative stress index (OSI) was determined using spectrophotometer and Rel Assay Diagnostics kits by measuring TAS and TOS in blood plasma. The cortisol level was measured using AIA-360 Automated Immunoassay Analyzer and ST AIA-pack Cortisol assays. Group I dogs' HR and f_R were elevated at T0 and T1, and group II dogs' –at T1 compared to physiological range. OSI and cortisol levels in group I dogs was higher at T1 compared to T0 ($P<0.05$). There was no significant difference in cortisol level between group I at T0 and group II at T1 ($P>0.05$). It might be concluded that dogs' longer stay in the novel environment without the owner induced significant changes in OSI and cortisol level, which could lead to slow wound healing and the occurrence of systemic diseases.

KEY WORDS: dog, oxidative stress, psychogenic stress

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Psychogenic stress is common in animals and it describes exposure to psychological or social challenges which result in disruption of psychological well-being. Negative psychogenic stress in a domesticated animal may be induced by separation from the owner, or exposure to a novel environment [37]. Stressful situations can impair animal's welfare and can have an influence on various organism systems, especially for hypothalamic-pituitary-adrenal axis (HPA) activation. Biochemical and molecular changes can emerge at the cellular level in response to exposure to stressful situations. During stress, body releases adrenaline and cortisol hormones. Adrenalin cause increase in heart and respiratory rate and cortisol suppress the immune system [3, 6, 10, 19, 22, 26, 27, 36]. These changes can affect reactive oxygen species (ROS) formation in the body what can lead to OS. Excess ROS amount attack biologically relevant molecules such as lipids, DNA, carbohydrates, and proteins, causing cell membrane and nucleus impairment, and form destructive products, such as lipid peroxides. DNA damage results in carcinogenesis and mutagenesis, while protein damage results in loss of enzyme activity [10, 19, 22, 29, 41, 42, 47]. Moreover, examination of OS occurrence due to psychogenic stress in dogs might have practical significance, both for the welfare of confined dogs, and possibly for better understanding of negative clinical outcomes (postoperative anemia, prolonged wound healing and prolonged postoperative recovery rates) which originate after surgeries. It is crucial to ensure patients safety before and during anesthesia, as OS level also elevates after anesthetics administration [24, 38, 41, 46].

To our knowledge, there have been numerous studies investigating the effects of novel environment and separation from the owner on the occurrence of psychogenic stress [13, 21, 44]. However, during these studies the authors did not investigate the influence of psychogenic stress on the occurrence of OS in dogs. We put forward the hypothesis, that long-term psychogenic stress induce significant changes not only in cortisol level but also in OS parameters (including plasma total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI)) in client-owned dogs. For this reason, the purpose of this study was to

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evaluate the influence of long-term separation from the owner in the novel environment on the occurrence of psychogenic stress and OS in dogs.

MATERIALS AND METHODS

Eighteen client-owned dogs, 10 females and 8 males, 14.9 ± 1.5 month, weighing 19.3 ± 0.5 kg, scheduled for ovariohysterectomy or orchiectomy surgery were used in this study. All dogs were categorized to be in I group according to the American Society of Anesthesiologists (ASA) classification, based on a physical examination, complete blood cell count, analysis of serum biochemistry, and thoracic radiographs. The study was carried out in compliance with the EU legislation. The procedures complied with the criteria given by the Lithuanian animal welfare regulations (No. B1-866, 2012; No. XI-2271, 2012) and the decree of the director of the State Food and Veterinary Service, the Republic of Lithuania (No. B6-(1.9)-855, 2017).

Nine dogs (group I) were confined to the kennel room in the veterinary clinic in the evening, 12 hr before the surgery (the dogs previously have never been isolated in the novel environment and separated from their owners), and other nine dogs (group II) were confined to the kennel room in the veterinary clinic 10 min before the surgery. Both group dogs were brought to the veterinary clinic 20 min before they were confined to the kennel room. During this time, physical examination, blood sampling for complete blood cell count and analysis of serum biochemistry, and thoracic radiographs were done. Owners were together with their dogs during these procedures.

Physiological parameters (heart rate (HR) (beats/min) and respiratory rate (f_R) (breaths/min)) were observed by auscultation. Dogs were considered to have tachycardia if their HR was higher than 100 beats/min. Tachypnea was characterized by a f_R that was higher than 30 breaths/min. The first observation of HR and f_R was performed 12 hr before the surgery (T0) for group I dogs, and the second observation-10 min before the procedure (T1) for group I and group II dogs. Group I dogs were taken out of the cage.

The housing in the individual cage in novel environment and separation from the owner were considered as stressors for dogs. Group I dogs were housed in individual cages, without food. Drinking water was available *ad libitum*. Group II dogs has been subjected to food restriction 12 hr before being admitted. The temperature of the kennel room was $19 \pm 1^\circ\text{C}$ and the air humidity was $50 \pm 10\%$.

Venous blood samples (3 ml) of the dogs were collected into heparin tubes via venepuncture from the jugular vein. Blood samples were taken 12 hr before the surgery for group I dogs (T0) and 10 min before the surgery for both group I and group II dogs (T1). Plasma samples were separated by centrifugation at 1,500 rpm for 15 min and were stored at -80°C until their test. The cortisol level ($\mu\text{g/dl}$) was measured using AIA-360 Automated Immunoassay Analyzer (Tosoh Bioscience, Inc., South San Francisco, CA, U.S.A.) and ST AIA-pack Cortisol assays (Tosoh Bioscience, Inc.). The total oxidant status (TOS) as well as the total antioxidant status (TAS) was determined using Lambda 25 UV/Vis spectrophotometer (PerkinElmer, Waltham, MA, U.S.A.) and Rel Assay Diagnostics kits (Mega Tip, Gaziantep, Turkey), following the manufactures' instructions [11, 12]. Oxidative stress index (OSI) was calculated as follows: $\text{OSI (arbitrary unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Eq/l}) / \text{TAS } (\text{mmol Trolox Eq/l}) \times 10$ [2].

The statistical analysis was performed using computer software SPSS 22. Averaged experimental results are reported as means \pm standard error of the mean. Cortisol levels, HR and f_R between group I at T0 and group II at T1 were compared by Independent *t*-test. HR, f_R , cortisol levels, TAS, TOS and OSI parameters in group I between T0 and T1 were analysed using paired *t*-test. The level of significance was set at $P < 0.05$.

RESULTS

Initial physiological and vital parameters of all dogs were similar and within normal range in both group I and II dogs. Animal age (group I -14.3 ± 1.2 months; group II -14 ± 2.2 months) and weight (group I -18.7 ± 0.6 kg; group II -19.6 ± 0.8 kg) did not differ between the groups ($P > 0.05$).

In the present study, HR and f_R were observed in dogs. All dogs of group I had increased HR and f_R compared to physiological range at T0 and T1 (HR: 135 ± 4 beats/min vs 136 ± 4 beats/min, respectively; and f_R 38 ± 2 breaths/min vs 39 ± 2 breaths/min, respectively). There was no significant difference between T0 and T1 in group I dogs in HR and f_R ($P > 0.05$). Group II dogs also had increased HR and f_R compared to physiological range at T1 (HR: 138 ± 8 beats/min; and f_R 42 ± 4 breaths/min). There was no significant difference between group I at T0 and group II at T1 in HR and f_R ($P > 0.05$).

The long-term duration of the preoperative time spent in the veterinary clinic was effective in inducing measurable psychogenic (group I and group II dogs) and oxidative stress (group I dogs) in dogs. TAS, TOS, OSI and cortisol values are represented in Table 1.

In the present study, cortisol level elevated in group I dogs at T1 compared to T0 ($P < 0.05$). There was no significant difference in cortisol level between group I dogs at T0 and group II dogs at T1 (Table 1) ($P > 0.05$). TOS and OSI levels increased ($P < 0.05$), while TAS level decreased in group I at T1 compared to T0 ($P > 0.05$). Paired *t*-test revealed differences in cortisol, TAS, TOS and OSI levels in group I dogs between T0 and T1 time points ($P < 0.05$).

DISCUSSION

A visit to a veterinary clinic may be stressful for the patient. Stressful situations are associated with physiological changes, increased cortisol and catecholamine production and oxidative stress [5, 13, 33]. Usually, dogs visit veterinary clinic due to annual vaccination, routine checks or for elective surgery. There are situations, when a dog can be separated from its owner in the

Table 1. Oxidative stress parameters and cortisol level of the dogs

Parameters	Dogs	T0 (12 hr before procedure)	T1 (10 min before procedure)	P value
TAS (mmol Trolox Eq/l)	group I (n=9)	0.47 ± 0.02	0.43 ± 0.02 ^{a)}	<0.05
TOS (μmol H ₂ O ₂ Eq/l)	group I (n=9)	4.33 ± 0.11	6.13 ± 0.10 ^{a)}	<0.05
OSI (arbitrary unit)	group I (n=9)	0.93 ± 0.03	1.43 ± 0.03 ^{a)}	<0.05
Cortisol (μg/dl)	group I (n=9)	2.50 ± 0.27	6.81 ± 0.34 ^{a)}	<0.05
Cortisol (μg/dl)	group II (n=9)	-	2.65 ± 1.42 ^{b)}	>0.05

The values represent the average ± standard error. a) Statistically difference compared to T0 (n=9) ($P < 0.05$). b) No difference in cortisol levels between group I at T0 and group II at T1 ($P > 0.05$).

veterinary clinic (before the surgery, etc.). Dogs confined in veterinary clinic cage room before surgery experience a wide range of psychogenic stressors, including not only separation from the owner and impact of novel environment, but also noise, unfamiliar people, unpredictability, constrained environment [3, 13, 18, 21, 35, 44]. It is very important to reduce psychogenic (do not leave the dogs in the veterinary clinic before surgery for long period of time) and oxidative stress of pre-operative hospitalized dogs under veterinary care, because it seems likely that the impact of these types of stress on clinical outcomes, such as postoperative anemia or prolonged postoperative recovery rates, is underestimated [38, 41].

In the present study we have showed, that long-term separation from the owner in the novel environment induced psychogenic stress and increased cortisol level in group I dogs. Cortisol has been used as a stress biomarker in both humans and animals [8, 15]. Our data correspond with other studies that had investigated psychogenic stress in rodents, primates and dogs. The studies with rodents and primates determined that isolation in the novel environment activates HPA axis [17, 30]. Tuber *et al.* [43], in their study also have proven that domestic dogs placed into a novel environment for 4 hr showed an increasing of plasma adrenocorticoid levels. According to Hennessy *et al.* [16], dogs sampled at their homes were found to have much lower plasma cortisol levels than did the comparison group of dogs sampled in the novel environment (county animal shelter) on the days 1–3 of the trial.

In the present study we have also shown, that stressors, but not daytime had an influence on fluctuations of cortisol levels in group I dogs. We compared group I (blood sample was taken in the evening) and group II (blood sample was taken in the morning) dogs blood plasma cortisol levels and did not find any significant difference ($P > 0.05$). Kolevska *et al.* in their study have not found diurnal rhythms in experimental (dogs used for experimental purposes on a long-term basis) and in working group (dogs used for various operations) dogs, but circadian rhythm was found in dogs with common daily routine with no physical and emotional load [23]. Takahashi *et al.* [40] did not confirm the diurnal cortisol secretion rhythm in their study, as well as Kemppainen and Sartin [20]. However, whether cortisol in dogs' blood shows circadian rhythm is controversial. Beerda *et al.* [4] have shown that cortisol levels change according to a circadian rhythm, having a peak in the morning hours decreasing gradually until the evening. Palazzolo and Quadri [31] have found that circadian rhythm is present in adult dogs only, while in puppies and old dogs no rhythmic fluctuations in cortisol secretion were found.

Reactions to stress are associated with increased secretion of hormones such as glucocorticoids, catecholamines etc., which change physiological parameters—accelerate heart and respiratory rate, increase cardiac output [34].

In the present study, HR was increased comparing to physiological range. Our findings are in agreement with those of other studies that investigated the effect of stress on the HR. Csoltova *et al.* [9], have shown that veterinary examinations produced acute stress responses in dogs with significant increases in HR. Also, increases in HR (136.4 ± 17.2 beats/min) and cortisol level (179.89 ± 91.87 nmol/l) were observed in dogs, which have been stressed while waiting in the veterinary clinic waiting room [32].

During our study, f_R rate was elevated comparing to physiological range in dogs. Increased f_R refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of ROS [7, 28]. Srithunyarat *et al.* [39] in their study have shown that dogs came for ovariohysterectomy had elevated f_R (92.6 ± 63.3 breaths/min) and cortisol level (174.6 ± 78.5 nmol/l) due to stress caused by novel environment (veterinary clinic).

In the present study, we also evaluated the effects of novel environment and separation from the owner on the occurrence of oxidative stress in dogs. Usually all types of stresses appear at the cellular level as oxidative stress that is characterized by decreased activity of antioxidant defense system and increased production of ROS. Our data showed, that long-term separation from the owner in the novel environment activated antioxidant defense mechanisms in dogs (increase in oxidants (TOS) level and decrease in antioxidants (TAS) level). Elevated oxidant-antioxidant ratio could occur due to activation of HPA axis, which caused different effects on physiological parameters (HR, f_R). According to Maksymchuk *et al.* [25] psychogenic stressors significantly altered oxidative stress markers in mice liver. In the human studies, it was shown that chronic stress exposure promotes oxidative damage through the activation of the HPA axis [1]. Also, there is some evidence, that behavioural stress increases oxidative damage to cell nuclei in humans [14].

This study provides valuable data on the impact of long-term (chronic) stress on OS in dogs. It might be useful for veterinary surgeons, as during surgery, anesthetics and surgical trauma also increase OS level, and higher than normal OS can have a negative influence on postoperative recovery rates and wound healing [24, 38, 45].

These findings support the hypothesis that long-term stress induced significant changes in OSI, which could lead to slow wound healing and the occurrence of systemic diseases.

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