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Research

Possible application of oxidative stress parameters for the evaluation of animal welfare in sheltered dogs subjected to different environmental and health conditions

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ABSTRACT

The objective of this study was to establish an additional prognostic parameter to assess animal welfare. We explored the validity of using the thiol antioxidant barrier (SHp) and determination of reactive oxygen metabolites (dROMs) as indicators of stress and welfare in sheltered dogs. The shelter is a stressful environment for a dog because of its unpredictable and uncontrollable nature. The dogs enrolled in the present study belonged to 2 types of animal shelters (shelter 1 and shelter 2) that differed for social and spatial restrictions for the dogs. Animals were tested for leishmaniosis and ehrlichiosis and divided into 4 groups on the basis of their negative (group A and B) or positive (group C and D) status and the shelter they belonged to. The Student t test showed significant differences on SHp concentrations between groups A and B (negative titers; different shelters) ($P < 0.0001$; $t_{(10)} = 11.08$), groups C and D (positive titers; different shelters) ($P < 0.02$; $t_{(7)} = 2.998$), and groups A and C (different infection status and same shelter) ($P < 0.004$; $t_{(8)} = 3.975$). Levels of dROMs showed significant differences between groups A and C (different infection status and same shelter) ($P < 0.03$; $t_{(8)} = 2.552$) and groups B and D (different infection status and same shelter) ($P < 0.02$; $t_{(9)} = 2.817$). The high dROMs values recorded in all dogs during the study may be because of the stressful environment of animal shelters, in general. The low SHp concentrations that we found in dogs from shelter 1 may suggest a highly stressful condition related to the poor social environment for interaction. SHp and dROMs may provide useful information about the responsiveness of sheltered dogs subjected to different environmental (social and spatial restrictions, management practices, and diet) and health (negative or positive status for leishmaniosis and ehrlichiosis) conditions and may suggest the possibility of establishing an additional prognostic tool for the assessment of welfare and health in sheltered dogs.

Introduction

Animal welfare is the physical and psychological well-being of animals. The term "animal welfare" is being used increasingly by corporations, consumers, veterinarians, politicians, and others (Hewson, 2003).

Several tools that measure the effects of the dog's appraisal of its environment and of its efforts to cope with it are required to establish welfare problems. Poor housing conditions, harsh training sessions, and an uncontrollable and unpredictable social environment are examples of situations that may seriously affect dog welfare. To establish the presence of stress and associated welfare problems in dogs, behavioral stress parameters are of special interest because they are usually measured easily and noninvasively. A variety of behavioral responses have been reported to occur during acute stress; panting, vocalizing, paw lifting, snout licking, lowering of the posture, and so on. However, adaptation of the animal to the stressors may render such indicators of acute stress useless for establishing chronic stress, and chronic stress commonly leads to welfare problems (Beerda et al., 1997).

The development and validation of parameters and instruments for animal welfare assessment is necessary. Recent research on domestic dogs has focused on the use of physiology in welfare

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assessment, using parameters such as cortisol levels, heart rate, and catecholamines and immunological measures to examine how the animal is coping with its environment (Hiby et al., 2006). Measurement of oxidative stress may allow some estimation of the psychological defense involved in the prevention of the appearance of correlated pathologies (Piccione et al., 2007).

Oxidative stress refers to the cellular injury and pathologic change that occurs when there is an imbalance favoring oxidants over antioxidants within a living organism (Soffler, 2007). An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed oxidative stress. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions (Sies, 1997). Oxidative stress occurs when the antioxidant defense system is overwhelmed by an increased oxidant burden or a reduced antioxidant supply (Kirschvink et al., 2008). Stressful conditions lead to an imbalance between oxidants and antioxidants in favor of oxidants at the cellular or individual level (Khadija et al., 2009). The alteration of oxidative balance, if not adequately restored by the antioxidant barrier, induces an oxidative stress with cellular damage (Trevisan et al., 2001), which makes the organism susceptible to disease (McCord, 2000).

The influence of age and aerobic exercise on oxidative status of dogs has been studied (Hinchcliff et al., 2000; Cardini et al., 2005; Dunlap et al., 2006; Pasquini et al., 2008; Cocca and Maglione, 2010). Various physiological measures have been used as welfare indicators in sheltered dogs, including catecholamines, cortisol, heart rate (Beerda et al., 1996), and immune function (Beerda et al., 1999). This study investigated whether oxidative stress parameters, like thiol antioxidant barrier (SHp) and determination of reactive oxygen metabolites (dROMs), were suitable indicators of animal welfare in sheltered dogs subjected to different environmental and health conditions.

Materials and methods

Animals and housing

For this study, we enrolled 21 adult mongrel dogs (2-11 years; 7 intact males, 1 neutered male, 11 intact females, 2 neutered females) housed in 2 types of animal shelters (shelter 1 and shelter 2) in Messina (Italy). The shelters differed in management practices as well as social and spatial restrictions to which dogs were subjected.

As is typical of most shelters, the population included strays and dogs abandoned by their owners for various reasons. All dogs were kept under natural photoperiod and a natural environmental temperature. All animals were subjected to clinical examination to assess rectal temperature, heart rate, respiratory profile (cough and nasal discharge), ocular discharge, appetite, fecal consistency, and behavior. Dogs that appeared aggressive (e.g., growling or displaying canines), timid (e.g., tucking the tail or retreating to the back of the cage), or emaciated/cachexic were excluded from the study. The dogs that passed the clinical examination were tested for leishmaniosis and ehrlichiosis and divided into 4 groups on the basis of their negative (group A and B) or positive (group C and D) status and the shelter they belonged to. At the time of the study, dogs from group A (n = 4) and group B (n = 8) were clinically healthy, free from external and internal parasites, and in good nutritional condition. Dogs that had tested negative (n = 12) had been in the shelter for 820 ± 40 days. Dogs from group C (n = 6) and group D (n = 3) were asymptomatic but serologically positive with a low titer 1/160 (Solano-Gallego et al., 2011) and showed no clinical manifestations of illness except a persistent flea allergy dermatitis. Dogs (n = 9) that had tested positive had been in the shelter for 210 ± 30 days.

Dogs from group A and C were housed in shelter 1, which complied with the maximum number of dogs allowed by the Italian law n.15/2000 (400 dogs) and was characterized by kennels that included an indoor section (3 × 2 m) and an outdoor section (3 × 4 m), as required by the Italian law (Anon, 1991, 2000). Dogs were fed by the same individual once a day with a high-quality commercial diet (crude protein [24%], crude fat [11%], crude fiber [2.7%], ashes [6%], calcium [1.2%], phosphorus [0.8%], vitamin A [14,400 UI], vitamin E [180 mg], copper [16 mg], and linoleic acid [3.7%]) in accordance with their body condition score, and water was available *ad libitum*. Dogs were taken for at least one 20-minute walk per day but were rarely engaged with toys.

Shelter 2 respected the maximum number of dogs permitted by law, but it did not possess suitable health allowances and dogs areas were not comparable to those required by law (closed boxes, open boxes, or enclosures). It was because shelter 2 did not meet the legal requirements that we were asked by the oversight authorities to assess the stress conditions. Dogs from groups B and D, housed in shelter 2, were maintained in a social group housing system of 8-10 individuals. They were fed by different individuals once a day with a balanced homemade diet (protein—cooked beef meat [source 24.9%], lipid—fat and vegetable oil [source 26.1%], carbohydrate—cooked rice and potatoes [source 46%], fiber—cooked peas and carrots [source 3%], without dietary supplement) in accordance with their body condition score, and water was available *ad libitum*. Dogs spent approximately 3 hours a day in interacting with humans and playing with toys. They were brushed once a month.

Blood sampling and analysis

Blood samples were collected from each animal's cephalic vein at the same time daily (beginning at 9:00 AM and ending within 60 minutes on different days in the 2 shelters). Samples were

Table
Mean values ± standard deviation of SHp, dROMs, and TP, expressed in their conventional units, with the relative and statistical significance obtained in all groups

Parameters	Reference ranges	Shelter 1			Shelter 2		
		Group A	Group C	Groups A + C	Group B	Group D	Groups B + D
SHp (μmol/L)	450-650[a]	102.94 ± 10.72	199.25 ± 46.89[b]	160.89 ± 61.21	323.81 ± 38.26[c]	299.18 ± 47.31[d]	317.09 ± 40.06
dROMs (U CARR)	56-91[e]	185.88 ± 29.07	137.12 ± 29.91[b]	161.98 ± 28.81	198.44 ± 44.91	120.56 ± 20.10[f]	177.20 ± 53.14
TP (g/dL)	5.4-7.1[g]	5.97 ± 0.83	6.61 ± 0.66	6.35 ± 0.76	6.72 ± 0.89	6.68 ± 1.46	6.70 ± 0.99

SHp, thiol antioxidant barrier; dROMs, reactive oxygen species; TP, total protein; CARR U, Carratelli Unit.
[a] Cocca and Maglione (2010).
[b] Significance: group C versus group A ($P < 0.004$).
[c] Significance: group B versus group A ($P < 0.0001$).
[d] Significance: group D versus group C ($P < 0.02$).
[e] Cardini et al. (2005).
[f] Significance: group D versus group B ($P < 0.02$).
[g] Kaneko et al. (1997).