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Comparison of stress level indicators in blood of free-roaming dogs after transportation and housing in the new environment

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ABSTRACT

Animal welfare is of increasing importance and minimizing the stress is one of its prerequisites. Transport and new uncontrollable or unpredictable social environment are stressful for dogs and can affect their welfare. Particular unpleasant situations for free-roaming dogs include their capture in public areas and caging during the transport, vehicle vibrations, traffic noise, unknown environment, unloading and restricted housing condition. The new environment for free-roaming dogs presents potentially stressful novel experiences, such as new surroundings, unfamiliar sights, sounds, smells and unfamiliar people and other dogs. The aim of the present study was to investigate the influence of transport and housing in the new environment on blood parameters (cortisol, glucose, cholesterol and triglyceride concentrations, and leukocyte count and neutrophil/lymphocyte ratio) of 40 free-roaming dogs. Glucose concentration, leukocyte and neutrophil counts, and neutrophil/lymphocyte ratio were significantly higher ($P < 0.001$; $P < 0.01$) after transport in comparison with the level of the same parameters detected in dogs after housing in the new environment. Cortisol, cholesterol and triglyceride concentrations and lymphocyte count were also increased immediately after the transport compared to the levels 24 h after when the dogs were housed in the new environment; yet, these differences were not statistically significant. Accordingly, study results indicate that the transport itself was more stressful for free-roaming dogs than their housing in the new environment.

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1. Introduction

Transport itself can be stressful for dogs (Bergeron et al. 2002). The typical cage environment also presents unpredictable external events, a lack or loss of control over the environment (Hennessy et al. 1998), limited opportunities for social interaction with humans (Coppola et al. 2006), presence of other dogs (Rooney et al. 2007) and high levels of noise (Blackwell et al. 2013), all of which can be highly stressful. All of these events elicit stress response, including endocrine changes in dogs. Noise is a physical stressor that can lead to behavioural and physiological responses, including intestinal problems and immunosuppression (Spreng 2000). Stress response may also result from either fear or anxiety (Casey 2002; Dreschel 2010). Hubrecht (1995) emphasized the importance for animals to have freedom in order to control their environment and to satisfy their social needs, both often restricted within kennel enclosures. Various physiological measures have been used as welfare indicators for dogs, including catecholamines (Beerda et al. 1996) and immune function (Beerda et al. 1999), but most recent research on mammalian species, including dogs, has focused on the glucocorticoids, mainly cortisol.

Cortisol is a biomarker commonly used for stress evaluation in dogs (Beerda et al. 1997; Coppola et al. 2006; Horvat et al. 2007; Haverbeke et al. 2008). It offers the advantage of being a sensitive and universally accepted indicator of stress, easily and inexpensively measurable by commercial kits. Glucose can also be used as the biomarker to assess the hypothalamic–pituitary–adrenal (HPA) axis response to stress. However, because this metabolite is also influenced by other factors, e.g. feeding and starvation, it is not as reliable as cortisol (Mormede et al. 2008). Nevertheless, its use in conjunction with cortisol determination may lend additional support to the assessment of the HPA axis response to stress.

During short-term stress, glucocorticoids improve fitness by energy mobilization (Möstl & Palme 2002). Accordingly, cholesterol and triglyceride concentrations can be useful indicators of response to stress.

This study aimed to determine stress response of free-roaming dogs after the transport and 24 h after housing in the new environment using a physiological measure (cortisol, glucose, cholesterol, triglyceride concentrations, leukocyte, neutrophil, lymphocyte counts and neutrophil/lymphocyte ratio).

2. Material and methods

2.1. *Animals and sampling*

The study was performed on 40 female free-roaming dogs, which were 2–4 years old and their body mass varied from 20 to 25 kg. All the tested bitches were involved in the programme of birth control of free-roaming dogs in Belgrade, Serbia. The dogs were captured in public areas in accordance with standard operative practice by the communal animal hygiene service

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'Veterinary Belgrade'. Each group travelled every other week for 30–45 min. The drivers and the travel route were the same for all days. Usually, a departure travel took place between 08:30 and 09:30 am. On arrival, dogs were housed individually in typical (1 × 1× 1 m) cages with possibility of visual, olfactory and auditory contacts between animals. In new environment, food was restricted and water was available ad libitum. The bitches were kept in cages until they were returned to public locations from which they were temporarily removed in order to be sterilized.

Blood (2 ml) was collected from the cephalic vein of each dog in disposable plastic syringes, containing heparin (1:1000) for the analysis of biochemical indicators of stress response (cortisol, glucose, cholesterol, triglyceride concentrations and leukocyte, neutrophil, lymphocyte counts and N/L ratio). The first blood sample was collected from each dog immediately after the transport. The second blood sample was collected from each dog 24 h after housing in the new environment. The blood was centrifuged at 3000 rpm for 5 min and stored at–20°C until assayed. Serum cortisol concentration was determined by enzyme-linked immunosorbent assay described by Ginel et al. (1998). Glucose, cholesterol and triglycerides were evaluated using spectrophotometric kits, glucose oxidase-phenol aminophenazone technique for glucose determination, cholesterol oxidase-phenol aminophenazone technique for cholesterol determination and glycerol-3-phosphate oxidase-phenol aminophenazone technique for triglycerides determination as previously reported (Tietz 1990). Differential white blood cell counts were measured using Siemens ADVIA 120 automatic cell counter.

2.2. *Statistical analyses*

Data were analysed by use of Graph Pad Prism software. Study results were statistically analysed and presented using mean value and standard deviation. Differences in cortisol and triglyceride concentrations, leukocyte, neutrophil, lymphocyte counts and N/L ratio were tested using non-parametric Mann–Whitney *U* test while paired *t* test was employed to determine differences in glucose and cholesterol concentrations immediately after the transport and 24 h after housing in the new environment.

3. Results and discussion

Psychogenic stressors have long been recognized as potent stimuli for HPA activation. Among the most effective of such stimuli are exposures to novel surroundings and separation from social attachment figures. During the transport and housing in the new environment, dogs are losing control and reduce predictability of future events what represents a stressful situation. Unable to achieve physiological behaviour during these procedures, free-roaming dogs could be frustrated, thus experiencing unpleasant emotional state (Dawkins 2000).

In the present study, level of cortisol concentration in free-roaming dogs immediately after the transport was higher compared to the concentration noted 24 h after the transport when the dogs were housed in new environment, but the difference was not statistically significant (Table 1). Although we were not always able to show statistical significance for all parameters between observed two treatments, there was a general tendency for a decrease in physiological responses associated with stress 24 h after the transport. There are several possible explanations why such differences were not significant. Firstly, these findings can indicate that differences in cortisol concentrations and stress responses should be consequences of temperament differences in free-roaming dogs. Secondly, individual differences like the effects of an unknown breed or earlier life experiences may further attribute to variability in stress responses. Genetics, age and social relations also modify the individual perception of a stimulus as a threat. Thirdly, the lack of statistically significant difference in cortisol level between the transport and the first 24 h in the new environment might be due to proximity of blood samples (less than 48 h). However, the results indicated that procedures such as loading, caging during transport, vehicle vibrations, traffic noise and unloading of free-roaming dogs that were captured in public areas by the veterinary service were more stressful in comparison with housing in the new environment. Transport presents a novelty for dogs, a mixture of different stressors, such as unusual experience, unfamiliar environment, high temperatures, noise and vibrations. It is well known that dogs show glucocorticoid elevations when exposed to novelty (Tuber et al. 1996). Additionally, some animals may suffer from motion sickness and vomit. According to Casey (2002), stress response in dogs during transportation was a result of fear or anxiety. Results of this study were not in accordance with the results of other authors (Fox et al. 1994; Siracusa et al. 2007) who observed higher cortisol concentration in dogs of known owners in the new environment compared to dogs immediately after transportation. Also, Part et al. (2014) observed that cortisol: creatinine ratio was higher in owned domestic dogs in the kennel than those in home environment. In the study of Frank et al. (2006), cortisol concentration increased during as well as after the transportation, indicating that transportation in general represents the stressful situation for dogs. Beerda et al. (1997) found that mean level of saliva cortisol increased from 3.6 ± 0.4 nmol/L before the transport to 37.4 ± 8.2 nmol/L immediately after arrival to the new environment. Also, Bergeron et al. (2002) observed significantly higher average salivary cortisol concentrations before (16.2 nmol/L) and after (14.8 nmol/L) air transport than the baseline levels, suggesting stress due to transport. These results indicate that procedures before and during transportation represent the stressors of high intensity. Furthermore, it is well known that the strays show a decrease in cortisol production within 10 days in a kennel environment (Hiby et al. 2006) and after 2 weeks in shelter (Hennessy et al. 2002).

Table 1. Biochemical parameters for 40 free-roaming dogs' stress due to transport and new environment; means ± SD (standard deviation) and significance.

Parameter	Immediately after transport	24 h after housing in new environment	*P* value
Cortisol (nmol/l)	66.14 ± 53.16	55.18 ± 44.90	NS
Glucose (mmol/l)	4.53 ± 0.97	3.63 ± 1.03	**
Cholesterol (mg/dl)	242.21 ± 86.73	228.31 ± 70.82	NS
Triglycerides (mg/dl)	77.72 ± 45.06	74.31 ± 30.67	NS

Note: NS = not significant.
$^{**}P < 0.001$.

Table 2. Differential leukocyte counts (×109 cells/L) for 40 free-roaming dogs' stress due to transport and new environment; means ± SD (standard deviation) and significance.

Parameter	Immediately after transport	24 h in new environment	*P* value
Leukocytes	15.09 ± 5.94	11.09 ± 4.83	*
Neutrophils	11.79 ± 4.84	7.56 ± 3.24	**
Lymphocytes	1.95 ± 1.02	1.78 ± 0.92	NS
N/L ratio	7.43 ± 4.20	4.87 ± 2.48	*

Note: NS = not significant.
*$P < 0.01$; **$P < 0.001$.

One of the main effects of cortisol is to ensure enough available energy in challenging situations by mobilizing glucose, fat and proteins (Feldman & Nelson 2004).

During the stress response, blood glucose concentrations increase. Cortisol and catecholamines facilitate glucose production as a result of increased hepatic glycogenolysis and gluconeogenesis (Desborough 2000). Gluconeogenesis is stimulated and glucose is made available for the heart, nervous system and skeletal muscles. In addition, peripheral use of glucose is decreased. This fact could be related to the results of the present study where at average glucose concentration in blood of the free-roaming dogs after the transport was significantly higher ($P < 0.001$) compared to glucose concentration obtained 24 h after the transport (Table 1). The lower concentration of glucose level of free-roaming dogs who were housed in new environment might be also due to restricted food in this period.

Since blood lipid levels are influenced considerably by hormonal changes of many types, an altered endocrine status during stress must be considered as a possible cause of changes in cholesterol and triglyceride concentrations. In the present study, higher cholesterol and triglyceride concentrations were detected in dogs immediately after transport comparison with the dogs 24 h after housing in the new environment but differences were not statistically significant (Table 1). These results indicate that stressors during transport are higher compared with those in new environment. According to Solin et al. (2013), experiments on rats showed that restraint stress is associated with an increase in plasma level of non-esterified fatty acids, total cholesterol, triglycerides, very low density lipoproteins (VLDL) and low density lipoproteins (LDL). In humans, higher serum cholesterol level detected in stressful conditions is due to hypovolaemia (Patterson et al. 1993). Koob (1999) noted that the main body response to stress is release of corticosteroids. Stress leads to hyperinsulinemia (Alvarez et al. 1989). As reported by Mayes (1988), hyperinsulinemia leads to increased activity of HMG-C0 reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase), which is responsible for increased cholesterol synthesis. Also, it has been known that acute stress induces transient reductions in triglyceride clearance in middle-aged men and women (Stoney et al. 2002) and in horses (Niedźwiedź et al. 2012).

The total leukocyte count was significantly lower ($P < 0.001$) 24 h after housing the dogs than that noted immediately after the transportation (Table 2). The observed leukopenia was composed of a significant decline in the number of neutrophils ($P < 0.001$) and minimal decrease in the number of lymphocytes without significant difference. This yielded a significantly lower ($P < 0.01$) N/L ratio (Table 2). These results can be explained by the effects of catecholamines and glucocorticoids. In response to catecholamines and glucocorticoids, neutrophils shift from the marginated to the circulating neutrophil pool but this neutrophilia might also be enhanced by the release of neutrophils from the bone marrow storage pool and the decreased emigration of neutrophils to the tissues (Siracusa et al. 2008). Beerda et al. (1997) found that the total leukocyte count to be significantly higher in dogs 3 h post-transport compared to pre-transport values attributing this finding to higher intensity of stressors for animals during the transportation than in the new environment. They also determined higher levels of cortisol, glucose, cholesterol and triglycerides as well as N/L ratio in the dogs post-transport.

4. Conclusion

The present study demonstrated the cortisol concentration with the levels of other physiological parameters such as glucose, cholesterol and triglycerides, leukocyte count and N/L ratio to be useful stress indicators for dogs. Although statistically significant difference in the level of cortisol, cholesterol and triglyceride concentrations and lymphocyte counts observed immediately after the transport and 24 h after the housing in the new environment was not recorded, all parameters showed a decreasing trend 24 h after the transport. It may be concluded that the transport itself presents more stressful event for free-roaming dogs than their housing in the new environment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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