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Variations of salivary cortisol in dogs exposed to different cognitive and physical activities

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ABSTRACT

Working dogs are gaining popularity for their ability to learn and perform tasks entertaining their human companions. For this reason, dogs are often subjected to various stimuli due to inter- and intra-specific interactions, environmental variations and effort required by different activities. In the present study, salivary cortisol was measured to monitor physiological response to different conditions. The first study was performed to assess the variability of salivary cortisol in dogs in usual environmental conditions. For this, salivary cortisol was measured in 10 dogs at home during three not consecutive days at three different times of the day and not significant variations between days and time of sampling were observed. In the second study, salivary cortisol was measured in dogs before and after Pointing Hunting (No. 5), Tracking for Ungulate Hunting (No. 6), Blood Tracking (No. 4), Agility Training (No. 6) and Animal Assisted Activities (AAA, No. 6). Salivary cortisol concentration significantly increased after the Pointing Hunting activity ($p < .05$), while salivary cortisol significantly decreased at the end of AAA session ($p < .05$). No significant differences in cortisol variations were observed for Tracking for Ungulate Hunting, Blood Tracking and Agility Training, before and after the activities. The response of cortisol suggests that the extent of hypothalamus–pituitary–adrenal axis activation varies between short high-intensity activities and endurance exercises. The measurement of salivary cortisol can support the trainers to evaluate the animal response to the stimulations.

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Cortisol; saliva; stimuli; exercise; environment; dogs

Introduction

The growing popularity in canine competitions has led to an increase focussing on dogs health status, cognitive skills and level of fitness. Dogs are trained to perform different tasks to entertain its human companion during sporting and hunting activities or to assist people as a service therapy dog. In these contests, dog may experience stress associated to exercise, social interactions, environmental factors, loud noises, exposure to novelties or high expectations of the handler (Beerda et al. 1998; Pastore et al. 2011; Shiverdecker et al. 2013). In order to understand the physiological response of the animals to exercise and related activities, many studies have been carried out to monitor the associated neuroendocrine and biochemical changes (Arokoski et al. 1993; Angle et al. 2009; Wakshlag et al. 2010; Yazwinski et al. 2013; Tharwat et al. 2014).

Among the biomarkers used to evaluate the physiological responses to exercise, salivary cortisol has been largely used in human and horse athletes (Schmidt et al. 2010; Pastore et al. 2011; Lippi et al. 2016; Vingren et al. 2016), but according to the recent review of Cobb et al. (2016) to a lesser extent in dogs. Cortisol is the main end product of the activation of hypothalamic-pituitary-adrenal (HPA) axis and its secretion show a sensitive response to environmental changes. However, cortisol concentration in blood can be affected by sampling procedure and sudden environmental changes. Thus, alternative sites of sampling have been recently investigated and the measurement of cortisol in saliva has gained popularity to monitor variations of physiological states (Bergamasco et al. 2010; Cobb et al. 2016; Colussi et al. 2016). Concentrations of cortisol in blood and saliva are highly correlated and its transfer occurs with a mean delay of 20–30 minutes (Dreschel and Granger 2009; Peeters et al. 2013). Furthermore, saliva is easy to sample and can be collected also by the owner or petter, without causing additional stress for the dog due to

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handling or changing environmental context (Hiby et al. 2006; Jones et al. 2014).

In the present study, we measured salivary cortisol in relation to different dogs' activities, like physical exercise and cognitive responses, to investigate their effects on the HPA axis stimulation. For this aim, salivary samples were collected from dogs performing Pointing Hunting, Tracking for Ungulate Hunting, Blood Tracking, Agility Training and Animal Assisted Activities (AAA).

Materials and methods

Recruitment of dogs

In the present study, all dogs were privately owned, more than 12 months old, clinically healthy, not in oestrus or pregnant, vaccinated and free from external and internal parasites, as assessed by a veterinary practitioner. Moreover, dogs were not under corticosteroid administration or drug therapy for at least one month before the sampling procedure. For each dog, the following information was also collected: date of birth, breed, size, sex (male, castrated male, female, spayed female), feeding schedule and type of food.

The aim of the study and the sampling procedures were disclosed to the owners and informed consents were obtained prior to any procedure. In order to avoid potential interference on cortisol response due to the presence of unknown people, saliva samples were collected by owners, trained on sample taking during a preliminary meeting. Each owner recorded the exact time of sample collection and signs of distress, if any. All procedures were performed in respect of the legislation on animal care (EU Directive 2010/63/EU) and the internal rules of University of Udine.

Study 1: baseline value of salivary cortisol

In the first study, 10 dogs were not involved in specific activities. Dog owners were asked to collect salivary samples for three not consecutive days (D1, D3 and D5): in the morning (MO), at the first interaction with human during the day (MD), and 30 minutes after the last interaction of the day with owner (EV), when dogs were rested and seemed relaxed. From each dog, saliva was sampled at the same time. The times of MO samples ranged from 6:30 to 9:30, those of MD from 9:30 to 15:00 and those of EV from 20:00 to 23:00.

Study 2: variation of salivary cortisol during activity

In the second study, dogs were already trained for the following five activities: Pointing Hunting, Tracking for Ungulate Hunting, Blood Tracking, Agility Training and AAA (Table 1). In order to obtain individual references of salivary cortisol concentration, a baseline sample (T0) was collected the day before the activity at EV time period from all dogs included in the analysis. T0 samples were collected at home 30 minutes after the last interaction of the day with them. Dogs of Pointing Hunting, Tracking for Ungulate Hunting and Blood Tracking were housed in kennels and dogs of Agility Training and AAA were housed at home. Moreover from all dogs, the second salivary sample (T1) was collected just before beginning of activity. This sample was collected to control the potential interference on the final results of the car transportation, from dogs home place to the place where performance was taken. Dogs involved in Pointing Hunting and Tracking for Ungulate Hunting conducted the activities with their owners in packs. Dogs involved in Blood Tracking and Agility Training conducted the activity individually, and those involved in AAA in couple, under owners control. In Pointing Hunting, Tracking for Ungulate Hunting and Blood Tracking, the samples of saliva from hunting dogs were collected during hunting sessions, which were fixed by hunters. Game animals were not specifically requested and involved for the study. Each activity occurred in one day and its briefly description, including the relative time schedule of salivary sampling, is described below.

Pointing Hunting: all dogs were in one pack and the session started at 8:00 and finished at around 12:00 (four hours). The pack carried out their ordinary hunting activities followed by their group handlers (the hunters). Dogs had to run quartering the hunting ground field appropriately and to point out typical

Table 1. Number, breed, sex and corresponding activity of dogs recruited for the study.

Activity	Breed	M/CM/F/SF	Total
Pointing Hunting	English setters	4/0/1/0	5
Tracking for Ungulate Hunting	Istrian short-haired hound	1/0/2/0	3
	Griffon nivernais	0/0/2/0	2
	Italian short-haired hound	0/0/0/1	1
	Total	1/0/4/1	
Blood tracking	Bavarian mountain hound	1/0/1/0	2
	Hanoverian scenthound	0/0/2/0	2
	Total	1/0/3/0	
Agility Training	Border collie	3/0/1/0	4
	Labrador retriever	0/0/1/0	1
	Crossbreed	0/0/1/0	1
	Total	3/0/3/0	
Animal Assisted Activity	Crossbreed	0/0/0/3	3
	Labrador retriever	1/0/0/0	1
	Shitzu	0/0/1/0	1
	Poodle	1/0/0/0	1
	Total	2/0/1/3	
Total dogs		11/0/12/4	27

M: male; CM: castrated male; F: female; SF: spayed female.

upland game birds, maintaining steadiness until the bird is flushed, after the gun shot of the handler the game supposed to be successfully finished. These steps were repeated for the entire duration of the hunting session. A T2 salivary sample was collected roughly at 15 minutes from the end of the session, that took place in a morning of November (weather T 10.6–11.9 °C relative humidity Rh 56–66% and wind speed 18–22 km/h, 46°15′58.5″N 13°04′35.0″E geographical coordinates). Time elapsed between T1 and T2 sample was similar for all dogs, i.e. 258 ± 3 minutes.

Tracking for Ungulate Hunting: the dogs in packs reached the woodland hill field travelling in their owner's car. All dogs arrived at the same time and T1 samples were collected 10 minutes after getting off the vehicle. Soon after, all dogs in packs were let free to smell the track of the deer, after around 30 minutes the ungulate was shot by handler/hunter. The wounded ungulate escaped to hide into the wood and dogs were hence put on the leash with his respective owner to track the blood scent of the escaping wounded deer for 30–50 minutes (mean 45 ± 14 minutes), followed by the hunter until they found the deer. About 15 minutes (16 ± 1 minutes) after the hunting is over, a T2 sample was collected. Time elapsed between T2 and T1 was 91 ± 14 minutes. The activity was carried out in the morning in February (weather T 7.1–8.4 °C relative humidity Rh 92–94% and wind 3–8 km/h, 46°07′32.0″N 13°28′16.2″E geographical coordinates).

Blood Tracking: activity was carried out on four tracks (one for each dog) of 1.5 km length, that were plotted on the ground by a third person with natural blood collected from the slaughter house. The blood was wrapped in a jute bag and left at the end of the tracks. Around the starting time of the activity (11:30 am), each leashed dog was conducted on his own designated field and just before the beginning of activity the T1a sample was collected. This sampling corresponds to the T1 sample of Tracking for Ungulate Hunting. Dogs were let free and a gunshot simulating the game wounding was used to initialise the trial; an extra sample was collected 10 ± 1 minutes after the shot (T1b sample). The track of blood scent was followed by the dog on a leash for about 60 minutes (mean 55 ± 8) and about 12 minutes (12 ± 2) after the end of the tracking, when the dog found the blood bag, a T2 saliva sample was collected. Time elapsed between T2 and T1a was 77 ± 9 minutes. The blood tracking trial was conducted in December (indicative weather T 7.8–9.9 °C, humidity U 69–76% and wind 6–10 km/h, 46°07′32.0″N 13°28′16.2″E geographical coordinates).

Agility Training: samples were collected from dogs fit for competing at the professional level in the large dogs category (65 cm jump obstacles for Border Collies), during a standard training session in a competition track. All dogs were familiar with the activity, arrived with the owner car to the field on the same time (from 8:30 to 8:50) and stayed in the car until the training started. The T1 sample was collected just before the beginning of activity of each dog, which consisted of about 3 minutes of free walking/running and 3–4 bouts of a full course, complete of 15 jump obstacles, like 'A' ramp, dog walk, tunnels, see saw, hoop and weaving poles. The T2 sample was collected 15 minutes after the end of the activity. The average time elapsed for the dogs from T2 to T1, including the warmup and 15 minutes after the end of exercise, was 40 ± 3 minutes. The sampled training sessions took place in North East of Italy (45°42′28.839″N 13°44′0.839″E geographical coordinates) in early morning of February (weather T 8.0–10.5 °C, humidity U 51–52% and Wind 39–41 km/h) and of September (weather T 24.3–24.9 °C, relative humidity U 51–52% and Wind 39–41 km/h).

Animal Assisted Activities: dogs trained to perform AAA were sampled during an indoor session in a kindergarten in North East part of Italy (46°4′15.85″N 13°14′4.485″E geographical coordinates) in May (T 21.3–21.9 °C, relative humidity U 20% and Wind 18 km/h) and in June (T 23.7–24 °C, relative humidity U 35–41% and Wind 7–12 km/h). Dogs arrived in the owner's car and were sampled (T1) just before beginning of the activity about 30 minutes after getting off the vehicle. The activity started at 9:30 right after the sampling. The session included a simple dog-child interactions under the supervision of the owner, which was a certified AAA dog handler. In these sessions, dogs were not on leash and groups were formed with 8–10 children, a couple of dogs, a teacher and the dog handlers. At the beginning of the AAA, children were seated on chairs and instructed by the dog handler how to interact with dogs. The human–animal interactions consisted of tactile and verbal contacts, gesturing with arms/hands, playing with dog's toys, holding and pulling the lead to handle the dog and some basic obedience commands. The activity lasted about 1.5 hours and the T2 sample was taken 13 ± 2 minutes after the end of the session. Time elapsed between T2 and T1 was 103 ± 2 minutes.

Salivary sampling procedure

To avoid contamination of samples and interference with the enzyme immunoassay (Dreschel and Granger 2009), dogs were refrain from drinking and eating

20 minutes before each sampling. Salivation was stimulated allowing dogs only to sniff food treats (Bennet and Hayssen 2010; Ligout et al. 2010). Owners collected saliva by gently placing swabs (Salimetrics, State College, PA) into cheek of the dog for approximately 90–120 seconds, a time adequate filling the swab with saliva. Samples were checked for visible contaminations 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®, 51.1534, Sarstedt, Germany), temporarily stored in an ice box before the final storage at −20 °C until the analysis. Before analysis, 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 EIA kit (Salimetrics, State College, PA) (Hekman et al. 2012). The antibody of the EIA kit is highly specific for cortisol and, according to the manufacturer's instructions, cross-reactivity with other steroids is lower than 0.57%. Samples were assayed in duplicate, using 25 µL of sample per well. The lower limit of sensitivity of the assay was 0.03 ng/mL. Average intra- and inter-assay coefficients of variation were less than 12% and 8%, respectively.

Statistical analysis

The data of type of activity, sex, age, breed, time of sampling and cortisol concentrations were stored in a spreadsheet using Microsoft Office Excel (2010, Microsoft Corp., Redmond, WA). In the first study, 10 dogs were sampled and 85 samples out of 90 were available, since the amount of saliva in five samples was not enough for analysis. The normality of distribution was assessed with the Kolmogorov–Smirnov test. A mixed model design with the fixed effect for day (D1, D3 and D5), the time of the day (MO, MD and EV) and the interaction was applied (SPSS 1997, SPSS Inc., Chicago, IL). A random factor of the subject repeated with day and time was included into the model. For the analysis in the second study, a total of 27 subjects were sampled and 93 samples out of 102 were available. Normality of distribution was assessed with the Kolmogorov–Smirnov test. Statistical analysis was performed using a mixed model procedure with the fixed effects of time of sampling (T0, T1, T2) and the random factor of subject repeated with time of sampling. Only for Blood Tracking, time of sampling was T0, T1a, T1b and T2. For both studies, adjusted means were calculated and significance between means were evaluated with Fisher's least significant difference (LSD) test.

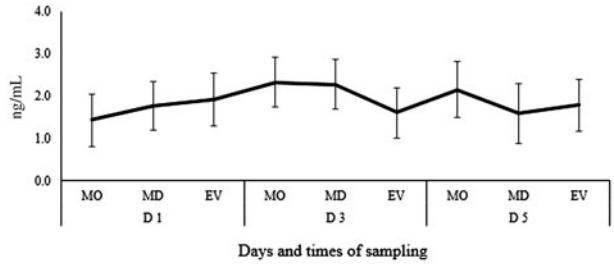

Figure 1. Variation of mean salivary cortisol in 10 dogs for three nonconsecutive days (D1, D3, D5) in three different moments of the day (MO, MD, EV). Notes: data were analysed with a mixed model with fixed effects for day and time of sampling and random effect of dog. No significant differences were observed. D1: sample collected on day 1; D3: sample collected after two days from day 1; D5: sample collected after four days from day 1; MO: sample collected at morning; MD: sample collected at midday; EV: sample collected at evening.

Results

Study 1: baseline value of salivary cortisol

The mean concentration of salivary cortisol of the 10 dogs enrolled in the study 1 (Figure 1), did not differ between the three days of sampling and between the time of the day (MO, MD and EV). The values, although variable, were below 3.0 ng/mL, without clear circadian variations.

Study 2: variation of salivary cortisol during activity

The 27 sampled dogs belonged to 10 different pure breeds or crossbreed (Table 1). Five dogs were sampled for the Pointing Hunting, six for the Tracking for Ungulate Hunting, four for the Blood Tracking, six dogs for the Agility Training and six for AAA. Among the not neutered dogs were 11 males and 12 females, and among the neutered dogs were four spayed females. Three spayed females practiced AAA and one spayed female practiced Tracking for Ungulate Hunting. Dogs were fed commercial complete dry food adequate to satisfy nutritional requirements. The effects of Pointing Hunting and Agility Trainer activities on cortisol concentrations are reported in Table 2. Salivary cortisol concentration significantly differed between times of sampling in Pointing Hunting ($p < .05$), increasing from T1 to T2 (4.61 ± 1.38 ng/mL and 16.33 ± 4.52 ng/mL, respectively). Instead, in Agility Training the variation of salivary cortisol was not significant, but the mean concentration of cortisol at the T2 showed the highest value (3.22 ± 0.51 ng/mL). In Blood Tracking activity (Table 2), the highest salivary cortisol concentration mean value was measured at T1b (3.04 ± 0.66 ng/mL) sampling time. The T2 sample,

Table 2. Variation of salivary concentrations of cortisol (ng/mL) measured in dogs and time elapsed between T2 and T1 for the different activities.

Activity Time of sampling	Cortisol, ng/mL Mean	 S.D.
Pointing Hunting		
T2 to T1 time elapsed, 258 ± 3 minutes		
T0 – Evening	1.30[b]	0.21
T1 – Before starting	4.61[b]	1.38
T2 – After the end of hunting	16.33[a]	4.52
Tracking for Ungulate Hunting		
T2 to T1 time elapsed, 91 ± 14 minutes		
T0 – Evening	1.57	0.10
T1 – Before starting	2.60	1.76
T2 – After the end of tracking	3.34	2.54
Blood Tracking		
T2 to T1a time elapsed, 77 ± 9 minutes		
T0 – Evening	1.27	0.36
T1a – Before starting	2.71	0.85
T1b – After gunshot	3.04	0.66
T2 – After the end of tracking	1.59	0.19
Agility Training		
T2 to T1 time elapsed, 40 ± 3 minutes		
T0 – Evening	2.48	0.43
T1 – Before exercise	2.80	0.28
T2 – After the end of exercise	3.22	0.51
Animal Assisted Activity (AAA)		
T2 to T1 time elapsed, 103 ± 2 minutes		
T0 – Evening	1.44[b]	0.10
T1 – Before activity	2.07[a]	0.16
T2 – After the end of activity	1.47[b]	0.15

Values with superscript letters a and b differ significantly, $p < .05$. T0 samples, for all activities were collected 30 minutes after the last interaction with the owner, when dogs were resting at home and apparently relaxed, according to the visual evaluation of the owner. Pointing Hunting: T1 samples were collected just before beginning of the activity and T2 samples were collected at 18 ± 3 minutes from the end of the session. Tracking for Ungulate Hunting: T1 samples were collected just before the beginning of the activity and T2 samples were collected 16 ± 1 minutes after the hunting was over. Blood Tracking: T1a samples were collected just before beginning of the activity, T1b samples were collected 10 ± 1 minutes after the gunshot and T2 samples were collected 12 ± 2 minutes after the end of the tracking. Agility Training: T1 samples were collected just before beginning of the activity and T2 samples were collected 15 ± 0 minutes after the end of the activity. AAA: T1 samples were collected just before beginning of the activity and T2 samples were collected 13 ± 2 minutes after the end of the session.

collected after the end of the activity, was not significantly different and numerically close to the baseline. Instead in Tracking for Ungulate Hunting, the highest salivary cortisol concentration, although not significantly different between T0 and T1, was measured at the end of the session, at T2 sampling time. Salivary cortisol concentrations measured for the AAA section (Table 2) significantly differed between times of sampling ($p < .05$), being higher at T1 (2.07 ± 0.16 ng/mL) in comparison to T0 (1.44 ± 0.10 ng/mL) and T2 (1.47 ± 0.15 ng/mL).

Discussion

Study 1: baseline value of salivary cortisol

The aim of the study 1 was to evaluate the extent of variation of salivary cortisol in dogs in a routine context and not stimulated for physical or psychological activities. For this reason, we asked the owners to collect the saliva on day 1 (D1), after day 2 (D3) and day 4 (D5) in three specific times of the day, in part following the sampling schedule adopted in a previous study (Sandri et al. 2015). Present results did not confirm the data obtained in the former study, which indicated a significant decrease of salivary cortisol in the evening sample. According to Giannetto et al. (2014), salivary cortisol shows a circadian rhythm, with peak around midday. This latter study was conducted in standardised conditions to evaluate the extent of circadian variations and sampling times were rigorously scheduled. Instead, in the present study, sampling times within the day were variable, to accomplish with owner availability to collect saliva during the day and, especially for the MD saliva swabs, a wide variation was obtained (from 9:30 to 15:00). The aim of our study was to understand if a single-shot sampling can be used as a baseline of salivary cortisol in a dog rather than studying circadian fluctuations or factor affecting cortisol variation. In a systematic review and meta-analysis of salivary cortisol in dog, Cobb et al. (2016) reported that salivary concentration measured early in the morning (from 6:00 am to 8:00 am) is significantly lower than those collected in the evening (from 6:00 pm to midnight) and during the day (from 8:00 am to 6:00 pm). In our study, the mean concentrations measured were not significantly different and numerically very similar, being 1.90 ± 1.59 ng/mL at MO, 2.20 ± 1.84 ng/mL at MD and 1.72 ± 1.88 ng/mL at EV. According to present and previous data (Giannetto et al. 2014; Glenk et al. 2014; Sandri et al. 2015), the late evening sample (EV) was considered appropriate as a baseline value. This sampling time was also the closest to the T1 sampling moment and this time was adopted in the study 2 as T0 value.

Study 2: variation of salivary cortisol during activity

In human and horse, physical exertion activates HPA axis leading to an increase of circulating and salivary cortisol concentration which is related to the performances (Arokoski et al. 1993; Schmidt et al. 2010; Lippi et al. 2016; Vingren et al. 2016), but less information is available for dogs (Cobb et al. 2016). Table 2 reports the cortisol response to exercises in dogs undergoing a short-duration high-intensity agility training or a long-duration high-intensity hunting section. In the Agility Training, either physical and psychological response occurs and, according to Pastore et al. (2011)

dogs can display several behaviours related to stress without a concomitant increase of salivary cortisol, probably due to the short duration of the exercise that activates only the sympathetic system. Instead, Pointing Hunting is an endurance exercise, which requires high amount of energy from body reserves. In this situation, the negative energy balance associated to strenuous physical activity can stimulate the secretion of cortisol (Royer et al. 2005). A significant increase of cortisol in blood has also been observed by Fergestad et al. (2016) after race in sled dogs and by Durocher et al. (2007) after a prolonged exercise in urine. Tracking for Ungulate Hunting and Blood Tracking (Table 2) engage lower physical activity and last shorter than Pointing Hunting (average of 91 minutes for Tracking for Ungulate Hunting vs. 77 minutes for Blood Tracking or around four hours for Pointing Hunting). In fact, in this study, we did not observe significant variations of cortisol concentrations for both these activities and only a numerically increase from T0 to T1 and T2 was observed for Tracking for Ungulate Hunting. Interestingly, for Blood Tracking, the increase of cortisol after the gunshot (T1b) could indicate that the sudden sound or the starting of session might impact most on variation of cortisol concentration rather than physical effort. Also, Beerda et al. (1998) have reported that a sound of blast triggers a significant increase of salivary cortisol, similar effect is obtained after a short electric shock, a falling bag, an opening umbrella and restraint. It is likely that also the excitement or anxiety before some expected event could have caused the increase of cortisol, as already reported by Angle et al. (2009), but no signs of distress were observed in the dogs of the present study after the gunshot. Of note was that the olfactory activities associated to tracking did not stimulate the HPA axis at an extent able to show detectable cortisol response in saliva. Shin and Shin (2016) reported that exposure of dogs to owner voice or odour leads, after a separation anxiety test, to a significant reduction of salivary cortisol. Recent researches showed that the areas stimulated by olfactory system in conscious dogs do not include hypothalamus and pituitary axis (Jia et al. 2014).

AAA does not require physical efforts, being a low-intensity exercise but it needs high psychological support. The significant increase of cortisol concentration before the activity (Table 2) can also explain with the excitement or anxiety before the event, as already reported by Angle et al. (2009). However, care should be taken to understand if the dog could have been stressed by the travel before his arrival to the therapy site. The return to the baseline level (T0) at the end of the session (T2) would indicate that the dogs were well trained and did not undergo stressing conditions in performing the interaction activities with the children. According to the observations of their handlers, the AAA dogs did not show particularly sign of distress. If dogs are approached and petted by people with whom they are not familiar, some discomfort can occur (Serpell et al. 2010; Colussi et al. 2016), but to participate to AAA activities, dogs should to remain calm and relaxed in variable situations and also under stressful conditions. As reported by Coppola et al. (2006) and, although with limited results, by Conley et al. (2014), the positive contact of dogs with human could contribute to maintain a low cortisol concentration. Furthermore, a positive human contact in a limited duration of assisted session can contribute to decrease the salivary cortisol level in dogs. Glenk et al. (2014) reported that in dogs trained for AAA and being leash-free during the therapy session, significant increase of salivary cortisol was not observed.

Conclusions

In conclusion, the results of this study showed that the salivary concentration of cortisol in dogs varies in relation to the type of activity. Cortisol variations depend on the extent of the exercise and on the level of alertness requested for the performance. However, further studies with bigger number of dogs are required to confirm the relationship between salivary cortisol concentration and factors as breed, predisposition to perform different activities and psychophysical preparation.

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Disclosure statement

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