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Cortisol concentrations in saliva of humans and their dogs during intensive training courses in animal-assisted therapy

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Summary

The cortisol concentration in saliva of dogs and their owners was measured as a parameter of disturbance, while they attended the 5 day training courses of the association "Animals as Therapy" in Vienna (Austria) to become "therapeutic teams" in animal-assisted therapy.

Samples were taken from 32 humans and their dogs (18 female and 15 male animals) during 3 training courses. The concentration of cortisol in saliva was measured using an enzyme immunoassay.

Among humans, increased values of cortisol were measured on the fifth day of the courses, which was the day of the final exams. Contrary, the levels of cortisol in saliva of the dogs did not differ significantly between individual days. The animals showed a non-significant trend to increased salivary cortisol levels during the first 3 days of the courses compared to the other days of their training and even had their lowest cortisol-median of all investigated days on the fifth day. This may be caused by the fact that the dogs got used to the new socio-ecologic circumstances that had bothered them at the beginning of their training. Neither among humans, nor among dogs significant differences in cortisol levels were measured regarding to sex and age of the participating subjects. Significantly more female than male dogs completed their training. This may lead to the conclusion that the demands on the dogs could be more easily fulfilled by females than males.

The results indicate that the training courses for working in animal-assisted therapy provided disturbance for the participating humans on the day of their exams. On average, no such disturbing situations could be detected among the dogs. We therefore conclude that this training is not stressful for the animals.

Schlüsselwörter: Hundebesitzer, Hunde, Ausbildungskurs, Belastung, Speichelproben, Kortisol.

Zusammenfassung

Cortisolkonzentrationen im Speichel von Menschen und deren Hunden im Rahmen der Ausbildung für „Tiere als Therapie"

In der vorliegenden Studie wurde geprüft, ob die fünftägige Ausbildung zu einem in der tiergestützten Therapie arbeitenden Team beim Wiener Verein „Tiere als Therapie" für Menschen und Hunde belastend ist. Als Parameter wurde die Kortisolkonzentration im Speichel gemessen.

Bei 3 dieser Ausbildungskurse erfolgte dreimal täglich eine Speichelprobennahme von 32 Menschen und ihren Hunden (18 weibliche, 15 männliche Tiere). In diesen Proben wurde die Kortisolkonzentration mittels Enzymimmunoassays gemessen.

Die Analysen ergaben, dass bei den Menschen die Kortisolkonzentration am letzten Kurstag (Tag der Prüfung) am höchsten war. Bei den Hunden hingegen konnten keine signifikant erhöhten Kortisolkonzentrationen am Prüfungstag im Vergleich zu den übrigen Tagen des Ausbildungskurses festgestellt werden. Die Hunde wiesen an diesem Tag sogar die durchschnittlich niedrigsten Kortisolkonzentrationen im Speichel während des gesamten Kurses auf. Demgegenüber hatten die Tiere an den ersten 3 Ausbildungstagen leicht, wenn auch nicht signifikant erhöhte Werte gegenüber denen der weiteren Kurstage, was auf die den Hunden anfangs noch fremden sozioökologischen Bedingungen zurückgeführt werden kann. Weder bei den Besitzern, noch bei ihren Tieren konnten signifikante Unterschiede in der Konzentration von Kortisol bezüglich ihres Alters oder Geschlechts gefunden werden. Signifikant mehr weibliche als männliche Hunde beendeten ihren Ausbildungskurs vollständig. Dies könnte darauf hinweisen, dass die an die Tiere gestellten Anforderungen von weiblichen Hunden eher zu erfüllen waren als von männlichen Tieren.

Die Ergebnisse weisen darauf hin, dass die Ausbildungskurse für die Arbeit im Bereich der tiergestützten Therapie für die Menschen am Prüfungstag eine gewisse Belastung darstellten. Bei den Hunden hingegen konnten keine signifikanten Belastungsperioden nachgewiesen werden.

Introduction

In the past, studies addressing the topic of animal-assisted therapy have mainly dealt with the evaluation of its effects on needy groups of patients (FINE, 2000). Other studies have shown that situations which are cumbering and unpleasant for humans may lead to physical and psychological signs of stress (SILVER u. WORTMAN, 1980; KATHOL et al., 1989; JØRGENSEN et al., 1990; HERBERT u. COHEN, 1993). Investigations on dogs indicate that similar causes may lead to a development of stress symptoms in those animals as well (STAFLEU et al., 1992; MASON u. MENDL, 1993; BEERDA et al., 1998). Stimuli that may initiate denying behaviour in dogs are for example restraint or limitation from something they want to do or have (KNOL, 1989; MUELAS et al., 1993), loud and sudden noise (BUENO et al., 1989; ENGELAND et al., 1990), or every kind of social punishment (SCHWIZGEBEL, 1982). These are all examples of stimuli that may also affect a dog during the training and later during the therapeutic work in animal-assisted therapy.

We wanted to investigate those levels of disturbances that affected dog owners and their animals, while attending training courses to become "therapeutic teams" in animal-assisted therapy.

The method used in this study - the analysis of physical and psychological disturbances via measurement of cortisol in saliva - has already established itself within natural sciences for several reasons: first, this non-invasive method reduces possible additional secretions of cortisol induced by the impact of sampling itself to a minimum (BEERDA et al., 1996; KIRSCHBAUM u. HELLHAMMER, 2000); second, the levels of cortisol in saliva are well correlated with the levels of plasma cortisol, both in humans (KIRSCHBAUM u. HELLHAMMER, 1989) and dogs (VINCENT u. MICHELL, 1992; BEERDA et al., 1996); and third, this method is as well applicable for the measurement of acute and chronic stress, as shown in dogs (BEERDA et al., 1998; BEERDA et al., 1999).

Material and methods

The association "Animals as Therapy" (Vienna, Austria) organizes animal-assisted programs in many different institutions (for example old people's homes, geriatric, psychiatric, drug-therapy wards, schools for mentally and/or physically handicapped children, schools for maladjusted children and adolescents). Besides this, it also offers training courses for private persons and their dogs, who wish to work within animal-assisted therapy as therapeutic teams.

Until the year 2004 the association offered special intensive-training courses for people with little time or from outside Vienna region or even abroad. These courses included 4 days of training, and subsequently 1 day of final examinations.

Subjects

Data were gathered during 3 consecutive intensive-training courses, the first from August 11th to August 15th 2002, the second from April 18th to April 22nd 2003, and the third from August 11th to August 15th 2003.

In total, 32 humans and 33 dogs participated in our survey (1 dog owner attended the training with two dogs). Among humans were 6 male and 26 female persons, among dogs were 15 males (3 gonadectomized) and 18 females (12 gonadectomized). The range of age was from 20 to 66 years among humans, and 1.5 to 10.5 years among dogs. Many different dog-breeds were represented (stated in alphabetical order): 2 Australian Shepherds, 1 Bernese Mountain Dog-Newfoundland-crossbreed, 1 Bobtail-crossbreed, 1 Border Collie, 1 Boxer, 1 English Cocker Spaniel, 1 Galgo Español, 1 German Short-Haired Pointer, 2 German Shepherds, 1 German Shepherd-crossbreed, 7 Golden Retrievers, 1 Greyhound, 1 Griffon-crossbreed, 1 Hovawart, 1 Husky-Collie-German Shepherd-crossbreed, 3 Labrador Retrievers, 1 Labrador-crossbreed, 1 Magyar Vizcla, 1 Miniature-German Shepherd-crossbreed, 1 Dutch Koiker, 1 Rhodesian Ridgeback, and 1 Tibetan Terrier. 1 small crossbreed could not be assigned to any breed.

Procedure of the intensive courses

Every training course started with a short entrance test for the dogs to select those animals that would surely not bring along all qualifications required. Special attention was given to the extent of socialization and the potential of aggression of each dog. During the rest of the day and the following 3 days practical units for the human-animal teams and lectures for the dog owners alternated. The lectures dealt with the topics geriatrics, psychology, animal-assisted therapy for children, dog behaviour, and first aid. During the practical lessons the instructors tried to examine the character of each dog, evaluated subordination and obedience towards the human owner, and checked the abilities of human and animal as therapeutic teams. The association demands a combination of excellent physical conditions (absence from endo- and ectoparasites, complete vaccination) with a salient character (absolute tolerance towards humans, dogs and other animal species of every age and sex, optimal socialization, very intense bond towards its owner, obedience on the level of the first companion dog title, resistence against stress, and total absence of aggression in any situation) from every therapeutic dog.

The afternoon of the fourth day was discretionary for each team.

During these 4 days 11 teams decided to drop out, because of private reasons, illness of dog owners or dogs, or because of disagreements with the instructors, or were discarded, mainly because of aggressive behaviour of the dog. This group included 9 female and 2 male dog owners, together with their 4 female dogs (1 gonadectomized) and 7 male dogs (1 gonadectomized).

The final exams during the fifth day of the intensive course included both a theoretical part for the dog owners and a practical part for the dogs. 21 teams passed the training units of the four preceding days and took part in the exams: 20 dog owners attended the theoretical part, but only the dogs from 10 teams were considered to be good enough to attend the practical part of the final exams. 1 team only participated in the practical part of the final exams, because the dog owner had already done the theoretical part during a former training course. All humans and dogs passed their exams. That means, out of 32 teams, only 10 finished their training with both the theoret-

ical and practical exam.

Among these 10 teams, 9 dog owners were female and 1 male, 8 dogs were female (6 gonadectomized) and 3 male (1 gonadectomized).

Sample collection

Saliva samples were collected each day of the courses three times a day by using Salivetten® (number 51.1534, Sarstedt, Wiener Neudorf, Austria): in the morning, immediately before the beginning of today's program, during lunch brake, and after the end of the daily program. The Salivetten® were left in the cheek pouches for about 30 seconds until they were saturated with saliva. Some dogs had problems producing enough saliva; in such cases several tricks were used to increase the production of saliva in the dog's mouth (like for example showing the dog something to eat or letting him smell something to eat). Afterwards the swabs were put back into the plastic tubes and placed on ice in the deep-freezer until the analysis.

All samples were centrifuged at 1.500 g for 10 minutes. Samples which contained less then 100 µl volume were excluded. Causes for less saliva in a sample were mainly too little production of saliva in the mouth during the collection of the samples or too early removement of the swab out of the cheek pouches.

The overall number of collected samples was 910; after centrifugation 606 samples were analysed (242 from course 1, 109 from course 2, 255 from course 3). A total number of 334 samples were collected from humans (53 from men, 281 from women), 272 from dogs (128 from males, 144 from females).

Analysis

The samples were measured using a "double-antibody biotin-linked enzyme-immunoassay" for cortisol (PATZL, 1990; PALME et al., 1996).

Statistical methods

Data were evaluated statistically by using SPSS computer-software. An evaluation for normal distribution using the Kolmogorov-Smirnov Test of Goodness to Fit showed that the data in this study did not underlie a normal distribution ($p \leq 0.001$). Thus Mann-Whitney-U Test, Wilcoxon-Signed-Rank Test, Kruskal-Wallis Test, and Friedman Test were used for further analysis. Box-Plot graphs were used for visualization.

In all statistical tests the probability of error was determined to be significant ($\alpha = 0.001$).

Results

Levels of cortisol in saliva measured during each day of the training courses

Tab. 1 shows the rapid decline of valid cases gathered per day, due to the increasing number of teams that dropped out during the course. Also striking is that the data of both dog owners and animals were subject to great distributions, especially those of the humans. Additionally, salivary cortisol concentrations of dogs were significantly lower than those from humans (Mann-Whitney-U Test, $p \leq 0.001$, Tab. 1).

Data were also analysed by using the Friedman Test. Among humans it showed that there were significant differences between the levels of cortisol in saliva measured per day ($p \leq 0.001$). These results were further investigated by using the Wilcoxon-Signed-Rank Test. We compared every single day among each other and found out that all days differed significantly from each other in its concentrations of salivary cortisol ($p \leq 0.001$). Outstanding in its data among humans was the fifth day, the day of the final examinations. On this day, all data collected, as well as their median, were outstrikingly high, also recognizable when comparing the minimum and maximum values (Tab. 1).

Among dogs no significant differences in salivary cortisol concentrations were measured between the days (Friedman Test, $p = 0.7$). A comparison of the data also showed that there was no comparable increase of the cortisol concentrations on the fifth training day. On the contrary, data even trended to be higher during the first 3 days of the courses and decline afterwards bringing up the lowest median on the day of the final exams. Anyhow, this was not statistically significant. Minimum and maximum values did not show any trends similar to those among the dog owners (Tab. 1).

Two Box-Plot graphs (Fig. 1, 2) visualize these results. Fig. 1 shows the graph of the dog owners, Fig. 2 the graph of their animals. Added in both graphs are the number of

Tab. 1: Number of valid and missing cases, median of cortisol concentrations (nmol/l) in saliva, and minimum and maximum values of cortisol concentrations (nmol/l) in saliva of humans and dogs, classified into each course day; combination of all 3 training courses

	Days of course	Number of valid cases	Number of missing cases	Median of cortisol in nmol/l	Minimum of cortisol in nmol/l	Maximum of cortisol in nmol/l
Humans	1	83	13	11.3	1.1	57.7
	2	80	16	12.9	2.3	58.5
	3	79	17	10.5	1.2	101.2
	4	61	35	13.5	2.3	52.9
	5	31	33	29.1	6.2	84.0
Dogs	1	70	29	2.8	0.6	16.6
	2	66	33	2.2	0.5	26.2
	3	73	26	2.4	0.4	38.9
	4	43	56	2.2	0.4	10.4
	5	20	46	1.9	0.5	18.5

Tab. 2: Number of valid and missing cases, median of cortisol concentrations (nmol/l) in saliva, and minimum and maximum values of cortisol concentrations (nmol/l) in saliva of humans and dogs classified into each time of the day; combination of all 3 training courses

	Day time	Number of valid cases	Number of missing cases	Median of cortisol in nmol/l	Minimum of cortisol in nmol/l	Maximum of cortisol in nmol/l
Humans	Morning	126	34	23.6	2.5	101.2
	Midday	115	45	12.1	1.2	76.6
	Evening	93	35	7.3	1.1	25.5
Dogs	Morning	99	66	2.6	0.4	16.6
	Midday	89	76	2.4	0.5	38.9
	Evening	84	48	2.0	0.5	26.2

Tab. 3: Number of valid and missing cases, median of cortisol concentrations (nmol/l) in saliva, and minimum and maximum values of cortisol concentrations (nmol/l) in saliva of humans and dogs, classified into each training course; combination of all days and day times

	Training courses	Number of valid cases	Number of missing cases	Median of cortisol in nmol/l	Minimum of cortisol in nmol/l	Maximum of cortisol in nmol/l
Humans	1	129	39	14.5	2.4	76.6
	2	58	12	19.7	6.2	101.2
	3	147	63	10.2	1.1	46.5
Dogs	1	113	55	2.7	0.4	26.2
	2	51	19	4.3	0.9	38.9
	3	108	116	1.4	0.4	10.4

cases used for each Box-Plot and the median calculated from all 5 days in each particular group to illustrate daily variations in cortisol secretion (median $_{humans}$ = 12.9, median $_{dogs}$ = 2.4).

Levels of cortisol in saliva measured at the three collection times a day

Tab. 2 offers data assigned to the 3 times each day samples were collected, for both humans and dogs. A comparison between humans and dogs showed that the number of missing cases of dogs was greater than the one from their owners.

The statistical analysis of cortisol concentrations indicated that for humans the values declined from the morning towards the evening: median, as well as minimum and maximum values of "midday" were below those from "morning", but were higher than those from "evening" (Tab. 2). A Friedman Test was used to confirm this trend statistically ($p \leq 0.001$). For further investigation the Wilcoxon-Signed-Rank Test was used. The results showed that the cortisol levels in saliva differed significantly between all 3 times of the day ($p \leq 0.001$).

The daily pattern of the dogs differed from those of their owners. Here only a little decline was seen among the medians and no trends were identified among the minimum and maximum values (Tab. 2). However, according to the Friedman Test, there were significant differences between the levels of cortisol in saliva collected at the 3 times of the days ($p \leq 0.001$). The Wilcoxon-Signed-Rank Test was applied to compare every time of the day with each other. The results showed that samples collected in the morning were significantly higher than those collected at noon ($p \leq 0.001$) and in the evening ($p \leq 0.001$). Yet there were no significant differences in the concentrations of salivary cortisol collected at noon and in the evening ($p = 0.43$). That means that morning samples of dogs contained significantly more cortisol than the samples collected during the rest of the days, when no significant fluctuation was measured.

The Box-Plot graphs (Fig 3, 4) provide a survey about the information given above. Fig. 3 shows the graph of the humans, Fig. 4 the one of the dogs. Added in each figure are the number of cases used for each Box-Plot and, again, the total medians of all days from humans and dogs (median $_{humans}$ = 12.9, median $_{dogs}$ = 2.4).

Levels of cortisol in saliva subdiveded into the 3 training courses

Finally, data were analysed according to the 3 training courses. As shown in Tab. 3, about thrice as many samples were collected in the first and in the third course than in the second course. Among the dog owners the drop-out rate did not fluctuate much between the courses, but among the dogs the drop-out rate was high in the third course. In both groups the medians of the second course indicated the highest cortisol levels, followed by the medians of the first and the third course. Similar findings were seen concerning the minimum- and maximum values of the 3 courses (Tab. 3).

To get more detailed information the Kruskal-Wallis Test was used to investigate whether the differences found in the concentrations of salivary cortisol between the 3 courses were significant. For both humans and dogs this conclusion could be made ($p \leq 0.001$). Afterwards the Mann-

Whitney-U Test was applied to compare the three courses with each other. Among the humans the results showed that although the first and the second training course did not differ significantly in their levels of measured cortisol ($p = 0.004$), the third training course provided significantly lower levels of cortisol in saliva than the other courses ($p \leq 0.001$).

Statistical analysis (using the Mann-Whitney-U Test) among the dogs showed that the concentrations of cortisol differed significantly between all 3 training courses ($p \leq 0.001$).

Residual Outcomes

Age did not have any statistically signficant influence on the levels of cortisol concentrations in this survey, neither among humans nor dogs. The Mann-Whitney-U Test was used to compare the levels of cortisol in saliva among several groups of ages, but no significant results could be found ($p = 0.28$).

Similarly no significant differences existed between the cortisol concentrations in saliva measured in male and female humans and dogs. Nevertheless, the Mann-Whitney-U Test indicated a trend towards slightly higher cortisol levels among women than men ($p = 0.02$). Among the animals, male dogs showed a trend to have higher levels of salivary cortisol than female dogs ($p = 0.011$). Gonadectomy of dogs did not have a statistical influence on the levels of salivary cortisol in dogs (Mann-Whitney-U Test, $p = 0.1$).

Neither in humans nor in dogs did the cortisol concentrations in saliva differ between those individuals, who dropped out of their course, or finished it with either one or both of the final exams. An analysis of the first four days of the courses with the Kruskal-Wallis Test did not calculate for either group significant results ($p_{humans} = 0.012$; $p_{dogs} = 0.449$). Anyhow, the Mann-Whitney-U Test showed that significantly more female than male dogs finished their courses ($p < 0.001$). Similar results were not found among the dog owners ($p = 0.334$).

Discussion

Cortisol concentrations from both humans and dogs showed high variations. A number of other studies confirm this fact, both for humans (KUNZ-EBRECHT et al., 2003, 2004) and for dogs (LAWLER et al., 1975; KOEPKE u. OBRIST, 1983; BEERDA et al., 1997, 1998; KING et al., 2003).

Reasons mentioned most frequently for individual differences among non pathological subjects are gender (GARNIER et al., 1990), age (PALAZZOLO u. QUADRI, 1987), earlier life experiences (MELZACK, 1954), menstrual cycle (BAO et al., 2004), and - among dogs - their breed (CORSON, 1971).

In the Master's Thesis by HAUBENHOFER (2003), which built the initial study for this paper, the author linked the data of cortisol in saliva collected during the training courses with the results of questionnaires the dog owners had to answer. The results showed that those teams that were used to a stressful every day's life (generated by family with children, job, and/or leisure time) dealt with the stressful parts of the training courses more easily and had on average lower levels of cortisol in saliva than those teams that were not used to stress.

Among the dog owners salivary cortisol concentrations differed significantly between the individual days of the training courses. This becomes manifest when looking at the data of the fifth day, the day of the final exams, followed by the fourth day, the day before the exams. The fact that all days provided significantly different levels of cortisol in saliva may be explained by another result of this study, already mentioned above - the great individual differences of cortisol concentrations among humans. The remarkably high levels of measured cortisol in saliva during the fifth day (together with its beginnings during the fourth day) may be interpreted as exam nerves (WEISS, 1989). Cortisol levels increase as parameters like controllability and decision latitude decline, as KARASEK (1979) explained in his "demand- control" model for work-related stress. A similar situation was given during the final exams of these courses. Adult human beings, who were mainly situated within the middle-classes of society were suddenly pushed back again into the position of learners. They were reprimanded by instructors who displayed all deficiencies and mistakes of them and their beloved dogs. Controllability and decision latitude were surely reduced in some way, especially during the practical final exam of the dog, when the owners had to rely on the skills of their dog and had hardly any influence on the denouement of the exam.

In this context it seems interesting that these exam nerves were obviously not transmitted from the dog owners to their dogs. Contrary, the levels of cortisol in saliva did not differ significantly between the single days among the dogs. The animals even had their lowest median of all 5 cortisol concentrations situated on the fifth day. The medians of the other 4 days lay above 2 nmol/l, but the median on the fifth day was 1.9 nmol/l.

A comparable study was done by HENNESSEY et al. (1997) and thus may offer a possible reason for our results here. The study was done in public animal shelters. The scientists measured the levels of plasma cortisol of dogs from the 1st to the 10th day of their stay in the shelter. The results indicated that the dogs had significantly increased cortisol levels during the first 3 days after their arrival in the shelter and afterwards showed a decline until the 10th day. They attributed these results to the new environment, contacts to unfamiliar dogs and humans, new daily rhythms, and new demands made on them. The initial situation and following results from our study seem similar. Thus many of the teams did not live in Vienna, some not even in Austria, these training courses stood for a complete change of everything the dog used to know and do. The dog was separated from his home, family and friends, brought into a new city, hotel room and training centre, exposed to unknown dogs, instructors and other dog owners, was subject to the time schedule of the training, and had to fulfil tasks he normally was not used to. These requirements could have been the reason for the increased levels of salivary cortisol during the first days of the training, when everything was still new and unfamiliar. During the ongoing of the training the dogs maybe got accustomed to their situation.

In general the cortisol levels of both humans and dogs complied on average with those basal and stress-increased cortisol concentrations in saliva found in other studies.

In the Master's Thesis by HAUBENHOFER (2003) the

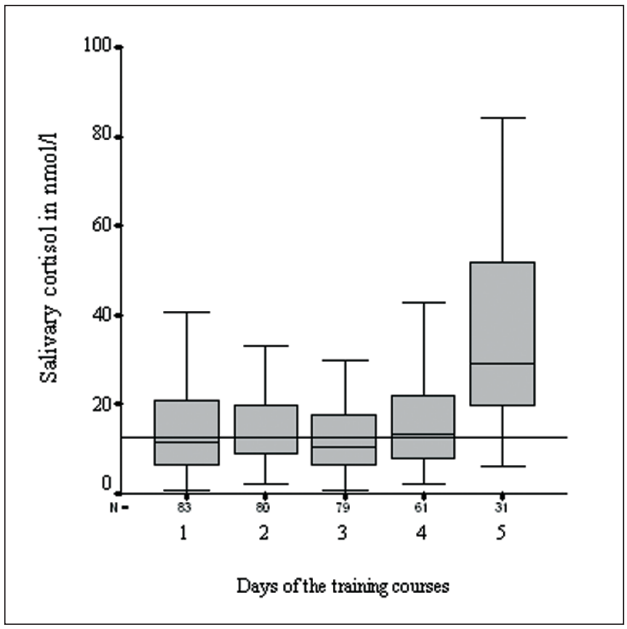

Fig. 1: Concentrations (nmol/l) of cortisol in saliva of humans measured each day during the training courses; data of all 3 courses are combined. Number of cases each day (n) and the median of all days (continuous line) are added.

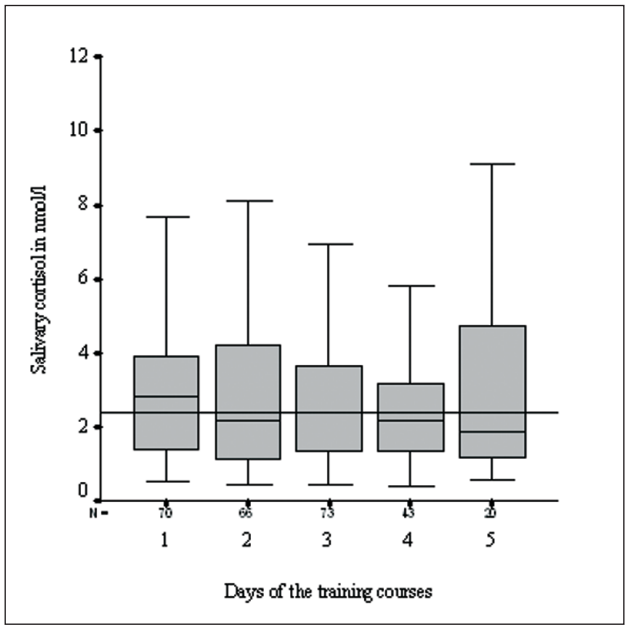

Fig. 2: Concentrations (nmol/l) of cortisol in saliva of dogs measured each day during the training courses; data of all 3 courses are combined. Number of cases each day (n) and the median of all days (continuous line) are added.

author compared samples of salivary cortisol collected during the training courses with samples collected from the same teams during leisure time. All teams had to collect saliva samples from themselves during an idle period of 2 consecutive days, for example a weekend.

The results of this comparison were consistent with the other results from this study mentioned above. Dog owners of those teams that were regularly exposed to stressful situations (by family, job or leisure time) secreted similar or even slightly higher levels of salivary cortisol during their weekend than during the first 4 days of their training courses. Cortisol concentrations as high as on the day of the final exams were, however, reached only in some cases. The results from the dog owners of those teams that were not used to stressful situations caused by normal life events were indeed different. These humans had continuously lower levels of cortisol in saliva during their weekends than during their training courses. Regarding to most of the dogs, concentrations of salivary cortisol were lower during weekends than during the first 3 days of their training courses. Levels of cortisol in saliva did not differ anymore when it came to the fourth and fifth day of the courses.

BEERDA et al. (1997) described a mean level of 3.6 ± 0.4 nmol/l as a basal cortisol concentration in saliva of Beagles during non stressed conditions, and 37.4 ± 8.2 nmol/l during stressful situations. In their following paper (BEERDA et al., 1998) they measured a mean basal cortisol level in saliva of 6.0 nmol/l for a group of dogs from different breeds. They exposed the dogs to several stressful situations, like pulling them on a leash, releasing a paper-filled bag from the ceiling, sudden noise, and electro shock. The stimulus “pull the leash” caused mean levels of salivary cortisol of 16.7 ± 12.1 nmol/l (due to one dog that showed extreme levels of 100.7 and 69.4 nmol/l), the loud noise caused an increase of cortisol up to 20.4 ± 4.5 nmol/l, the falling bag caused 18.7 ± 6.1 nmol/l, and the electric shock caused 15.5 ± 4.6 nmol/l. The 2 other tested stimuli - forcing the dog down on the floor, and opening an umbrella in front of the dog - caused no significantly increased cortisol secretion in the dogs. The scientists explain this to the predictability of stimuli used (BEERDA et al., 1998).

In their study about the evaluation of fear in dogs KING et al. (2003) determined the saliva cortisol concentrations of 108 dogs of various breeds, sex, and experience before and after 4 different tests that were thought to cause fear in dogs. The average concentration of saliva cortisol before the tests was 2.8 ± 3.8 nmol/l, while the mean concentration after the tests was 4.7 ± 5.6 nmol/l.

KUNZ-EBRECHT et al. (2004) investigated the salivary cortisol levels of humans during the first hour after waking up compared to the levels during the rest of the day. The mean level of cortisol in saliva directly after waking up in the morning averaged 18.9 ± 10.5 nmol/l, increasing to 28.0 ± 13.6 nmol/l 30 minutes later. The average cortisol levels in saliva during the rest of the day ranged in men from 7.52 ± 2.8 nmol to 9.65 ± 4.1 nmol/l and in women from 6.53 ± 2.0 nmol/l to 7.59 ± 2.6 nmol/l, contingent upon several socioeconomic parameters.

More causation for discussion in the scientific literature offers the existence of a daily rhythm of cortisol secretion in dogs. Among humans this question seems accepted in support of a daily rhythm. In humans, about 8-25 mg of cortisol are excreted every day (GRIFFIN u. OJEDA, 1996). An increase of cortisol production 3-5 hours after falling

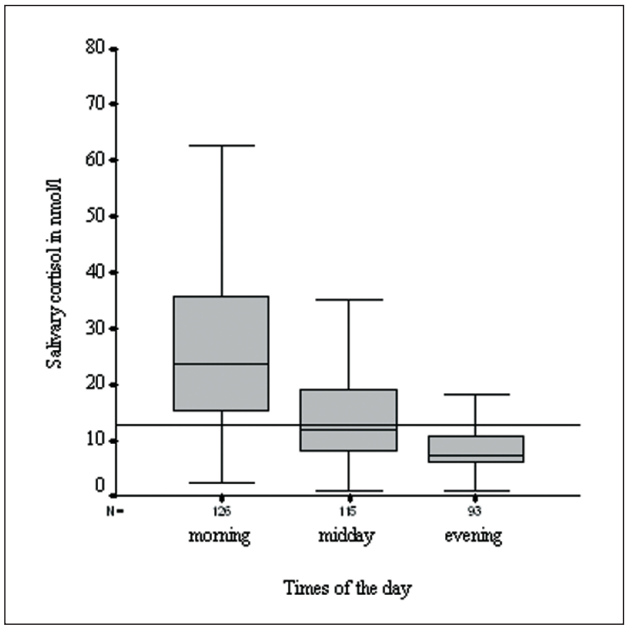

Fig. 3: Concentrations (nmol/l) of cortisol in saliva of humans assigned to the time of the day during the training courses; data of all 3 courses are combined. Number of cases each day (n) and the median of all days (continuous line) are added.

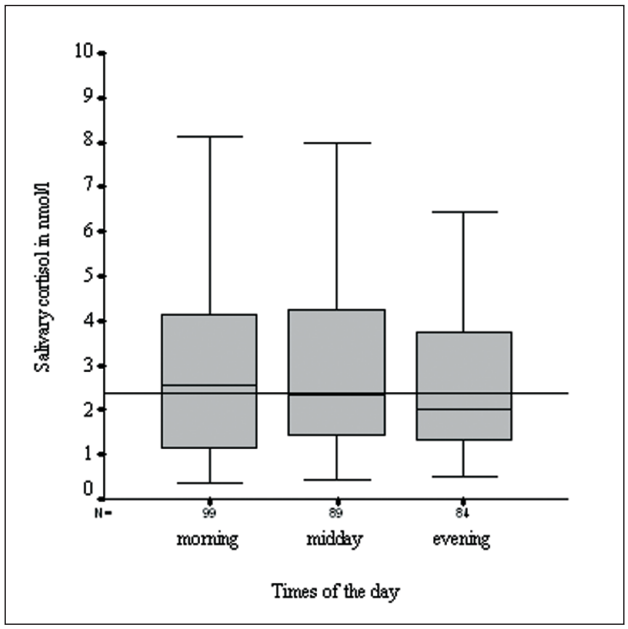

Fig. 4: Concentrations (nmol/l) of cortisol in saliva of dogs assigned to the time of the day during the training courses; data of all three courses are combined. Number of cases each day (n) and the median of all days (continuous line) are added.

asleep with a great peak in the morning about 1 hour after waking up can be noticed. During the rest of the day the cortisol concentration decreases again to reach its minimum a few hours before and after falling asleep again (HADLEY, 1996; KUNZ-EBRECHT et al., 2004).

In dogs the literature is less uniform. Some studies showed that the daily secretion of cortisol underlies similar rhythms as in humans (RIJNBERK et al., 1968; KOLEVSKÁ et al., 2003). Other authors claim that dogs loose their daily rhythm of cortisol secretion at an age of about 10 months (TAKAHASHI et al., 1981; KEMPPAINEN u. SARTIN, 1984; KOYAMA et al., 2003). This study reinforces the theory that dogs have increased concentrations of salivary cortisol in the morning. Nevertheless we cannot take for sure that the increased levels in the morning derived from a natural daily rhythm in dogs that leads to an increased production of cortisol in the morning. Saliva samples were collected when the dogs already were within the training centre and therefore exposed to all new and unknown socioecologic parameters mentioned above. Thus we cannot discriminate whether the increased levels of cortisol in the morning arose from a natural daily rhythm or from the situation of the training course itself.

Causation for the significantly different levels of averaged cortisol in saliva between the 3 training courses evaluated is speculative and hardly to evaluate thus the number of direct and indirect socioecologic parameters is too big. That also applies to the result that all 3 courses provided significantly different results among the dogs, but only between course 3 and the other 2 courses among the humans (although the trend remained the same in both groups). We can only mention that all courses were extremely individual both in their initial situation and their processing. This may have caused the observed differences.

The fact that no differences were found between the cortisol concentrations in saliva of males and females, between younger and older individuals, or rather between individuals who finished the training courses and those who dropped out, may be due to the small sampling size. Further investigation dealing especially with this topic would be advisable.

Yet interesting for the future of the training courses and the association “Animals as Therapy” may be the result that significantly more female dogs completed their training compared to the males. Certainly, also in this case, several parameters could have led to this result and discussion at this point will surely stay speculative. Maybe female dogs were more likely to fulfil the demands made on them because of their sex-specific character and behaviour.

Future investigation may deal with the evaluation of cortisol levels in human-dog teams during their therapeutic work in animal-assisted therapy to gain related information and possibilities of comparison.

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