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ASSESSING STRESS LEVELS IN THERAPY DOGS DURING ANIMAL-ASSISTED ACTIVITIES FOR CHILDREN ON THE AUTISM SPECTRUM

EVALUAREA NIVELULUI DE STRES AL CÂINILOR UTILIZAȚI ÎN TERAPIILE ASISTATE DE ANIMALE LA COPII CU TULBURARI DIN SPECTRUL AUTIST

Ana Maria ZAGRAI (MAIEREAN)[1)], D.C. COTOR[1)], G. ZAGRAI[1),3)], Aureliana GRAMA[3)], Alina Maria IONESCU[2)], G. COTOR[2)*)], A. DAMIAN[1)]

ABSTRACT | REZUMAT

The effects of stress on human and animal health and the methods of reducing it are intense topics addressed by researchers in the field. It has been known for a long time that stress in growth and transport can dramatically affect the health of animals, and stress is recognized as a factor in diseases that occur naturally in humans. The aim of our research was to assess the stressful effect of dog-assisted therapy activities on dogs in children with autism spectrum disorders. We initiated this approach by evaluating the haematological changes induced by these activities in the dogs' bodies. In our experiment we used 10 dogs (7 females and 3 males) of different breeds that ranged in age from 3-7 years. The dogs used in the experiment formed a uniform sample in terms of maintenance status. Haematological parameters were determined using a ProCyte Dx Hematology Analyzer. The data obtained were statistically analyzed, calculating the Mean and Standard Deviation (SD), using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated using the t-test (Student) using the Microsoft Excel application. We found that the specific activities of canid-assisted therapy, in the case of children with autism spectrum disorders, determined a significant increase in haematocrit, haemoglobin, and the number of red blood cells in the blood of dogs used in therapy, indicating a polycythaemia toning effect following eustress. The share of lymphocytes in the blood of the dogs used in therapy decreased, resulting in a confirmation of the eustress condition induced by the activity in which the therapy dogs were engaged. The eustress generated by canid-assisted therapy activities had a positive effect on the general condition, specific behaviours and haematological profile in the dogs used in the experiment.

Keywords: stress, autism, haematocrit, leukocytes, therapy dogs

Efectele stresului asupra sănătății oamenilor și animalelor și metodele de reducere predominante a acestuia reprezintă un subiect intens abordat de cercetătorii din domeniu. Este un fenomen cunoscut de mult timp ca stresul în creștere și transport poate afecta în mod dramatic sănătatea animalelor, iar stresul este recunoscut ca un factor al bolilor care apar în mod natural la oameni. Scopul cercetărilor noastre au vizat evaluarea efectului stresant pe care îl au asupra câinilor, activitățile specifice terapiei asistate de câini, in cazul copiilor cu tulburări din spectrul autist. Am inițiat acest demers prin evaluarea modificărilor hematologice induse de aceste activități, în organismul câinilor. În experimentul nostru am utilizat 10 câini (7 femele și 3 masculi) din rase diferite care au avut vârste cuprinse între 3-7 ani. Câinii utilizați în experiment au format un eșantion uniform din punct de vedere al stării de întreținere. Parametrii hematologici au fost determinați cu ajutorul unui analizor ProCyte Dx Hematology Analyzer. Datele obținute au fost analizate statistic, calculând-se Media și Deviatia Standard (SD), folosind aplicația Microsoft Excel. Totodată s-a calculat semnificația statistică a diferențelor dintre loturi cu ajutorul testului t (Student), folosind aplicația Microsoft Excel. Am constatat că activitățile specifice terapiei asistate de canide, în cazul copiilor cu tulburări din spectrul autist, au determinat creșterea semnificativă a hematocritului, hemoglobinei și a numărului de hematii în sângele câinilor utilizați in terapie, indicând un efect tonifiant policitemic, consecutiv eustresului. Ponderea limfocitelor în sângele câinilor utilizați în terapie a scăzut, de aici rezultând o confirmare a condiției de eustres induse de activitatea în care au fost angajați câinii de terapie. Eustresul generat de activitățile de terapie asistata de canide a avut un efect pozitiv asupra stării generale, comportamentelor specifice și a profilului hematologic, la câinii utilizați în experiment.

Cuvinte cheie: stres, autism, hematocrit, leucocite, câini de terapie

The effects of stress on people and animals' health, along with the predominant methods to reduce stress, represent a subject that researchers in the field often approach. It has been long known that stress during upbringing and transportation can dramatically affect animals' health, and stress is a recognized factor of diseases that naturally occur in people (15, 21).

The general adaptation syndrome, which is the result of short-term exposure to low- or medium-intensity stress factors, is generally known to have a posi-

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
2) University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
3) Saint Sylvester Secondary School, Bucharest, Romania
*) *Corresponding author: cotorg@yahoo.com*

tive effect by inducing eustress and improving the body's adaptive capabilities (2, 5, 16, 23). Exposing animals to powerful stress factors or over a long period of time may, in turn, induce distress. This has negative implications that may lead to a pathological evolution (1, 18, 19). Responding to stress is a normal part of daily life and it only becomes harmful when it's triggered too intensely or for too long (25, 22). The peripheral expression of the response to stress is modulated through two systems, the sympathetic-adrenal medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis.

Emphasis on animal welfare has significantly increased in the last few years. This includes the welfare of animals involved in activities designed to physically and psychologically improve people's health, such as Animal-Assisted Therapy (AAT).

Scientists involved in research, education, and the practice of animal-assisted activities (AAA) and of animal-assisted therapy (AAT), such as the International Association of Human-Animal Interaction Organisations (IAHAIO) and the American Veterinary Medical Association (AVMA), have published general guidelines and best practices to help ensure the welfare of therapy animals. IAHAIO's White Paper (2018), called "Definitions for animal-assisted intervention and Guidelines for wellness of animals involved in AAI" states that animals should be "in good health, both physically and emotionally and that [they] enjoy this type of activity". Therapy animals are asked to perform different activities, such as coming into contact with unknown people in an unknown environment (13, 20), and there's a possibility that these interactions may cause stress for therapy animals (24). During AAA sessions, dogs are exposed to new things, such as sudden noises, wheelchairs, and unusual substrate (7, 8). Moreover, inadequate environmental phenomena, high temperatures, lack of space, and clients' ages have been described as potential factors that negatively impact therapy dogs' wellness (12). As the use and consistency of AAA expands, so does the investigation of the effects of these activities on the health of therapy animals. Nevertheless, there are few studies focused on the possible impact of AAA sessions on the wellness of therapy dogs and existing studies offer mixed results (7). This type of therapy is being used more often as an additional means of therapeutic intervention for patients with various forms of psychological trauma or psycho-behavioural disorders (9). According to research, these treatments have yielded positive results that have encouraged their application on a broader scale. Disorders on the autism spectrum in children are among the psycho-behavioural disorders that can be treated with dog-assisted therapy (14). Measuring stress in animals is complicated (17), and understanding the impact of AAA on the health of therapy dogs is a true challenge. Wellness indicators also vary from study to study, including saliva, faeces, urine, or hair cortisol; heart rate (HR); questionnaires; interviews; behavioural observation; and cognitive tests. Given the wide range of frameworks and methodologies, some of these studies have presented habit changes that are generally associated with negative mood (high cortisol levels, stress-related behaviours), while others have found no significant changes in emotional indicators or have noted changes associated with positive moods (7).

Recent studies have shown the effects of stress on certain haematological parameters.This has increased the available range to evaluate the intensity of stress. This allows us to highlight that stress induces changes such as: an increase in red blood cells, the haematocrit and haemoglobin (5, 10, 11). Stress also induces changes of the leukogram, such as: an increase in white blood cells, neutrophils and a decrease in lymphocytes (2, 6, 18, 19). Here the specific effect of stress on the neutrophils/lymphocytes ratio becomes obvious, as it increases. Therefore, the neutrophils/lymphocytes ratio has become an efficient method in evaluating stress in mammals (19).

Since acute and chronic stress is associated with neutrophilia and lymphopenia, an increased neutrophils: lymphocytes (N:L) ratio was used as a stress marker in human and veterinary studies, including studies on dogs (3, 4). Since the N:L ratio appears a few hours after the stress inducer is present, it can be used to measure stress levels in dogs.

Our research focused on evaluating the stressful effect on dogs caused by activities specific to dog-assisted therapy in children with disorders on the autism spectrum. We initiated this research by evaluating the haematological changes induced by these activities in dogs' bodies.

METHODS AND MATERIALS

We used 10 dogs of different races for our experiment (7 females and 3 males), as outlined in Table 1.

Table 1

Table with the animals used in the experiment

Animal no.	Lot	Breed	Gender	Age (years)
1	1	Golden retriever	Female	3
2	1	German Shepherd	Male	3
3	1	Golden retriever	Female	7
4	1	Labrador	Female	4
5	1	German Shepherd	Female	5
6	2	German Shepherd	Female	3
7	2	Golden retriever	Female	4
8	2	Beagle	Male	6
9	2	Golden retriever	Female	5
10	2	Beagle	Male	4

The 10 dogs were aged between 3 and 7 years old. The dogs used in the experiment provided a uniform sample in terms of upkeep. The animals were fed dry food in quantities between 17-25 grams/kg/day. They were given water freely.

These animals were part of a group of animals used for treatments of children with disorders on the autism spectrum via animal-assisted therapy, applied by therapists from a specialised centre in Ilfov County.

The experiment was undertaken with written consent from the owners of the animals. We also had consent from the parents of the children involved in the experiment. The experimental procedures we imagined didn't affect the animals' wellbeing. Having said that, the only experimental intervention was taking some blood samples. We took these while respecting the norms to ensure the dogs' wellbeing.

The dogs used in the experiment were split in two experimental lots:

- Lot 1 (baseline), made out of 5 dogs (4 females and one male);
- Lot 2 (experimental), made out of 5 dogs (3 females and 2 males).

During the length of the experiment, in between physical activities that followed the treatment sessions, the dogs were kept in the treatment centre's dog kennel. The animals from lot 1 were held there for 4 days. The dogs from this lot were cared for during all this time by the centre's staff, and were not removed from the cages and paddocks where they were normally kept. The dogs from lot 2 were used for 4 days for activities specific to canine-assisted therapy for children with disorders on the autism spectrum. The dogs from this lot were cared for and supervised by the centre's staff during these activities and during the rest of the time. A therapist was also present during the therapeutic activities specific for assisting children with disorders on the autism spectrum. The dogs from lot 2 undertook therapeutic activities daily for 2 hours (between 10:00 and 12:00) during 4 days. The exercises they did consisted of the following activities: coming into contact with the patient, socialising and adapting to the child under treatment, heeling, running in tandem, and playing frisbee and other pet toys.

At the beginning of the first and fourth day of the experiment, blood samples were taken from the animals. Taking the blood samples is a work that has stress potential. As such, this was undertaken by the veterinary staff of the centre that supervised the canines daily. The staff are acquainted with the animals from the centre and, as such, the stress induced by blood drawing was significantly reduced.

Moreover, given the fact that both lots undertook the same potentially stressful procedure and the stress levels induced were similar, we conclude that the comparisons between the two lots are valid.

The blood samples were taken from the external saphenous vein and stored in heparinised 5 mL tubes. The samples were used to assess the following haematological parameters: haemoglobin, haematocrit, number of red blood cells, number of white blood cells, as well as the proportion of neutrophils and lymphocytes within the leukocyte formula.

The aforementioned parameters were determined with the help of a ProCyte Dx Haematology Analyzer.

Throughout the 4 days of the experiment, between 12:00 and 10:00 (the timeframe when all the animals from the experimental lots were kept in their respective boxes and paddocks in the dog kennel), the animals were under ethological supervision, which resulted in ethograms. The behaviours that were being investigated were as follows: the amount of food intake; the length of sleep periods; the length of self-grooming periods; the presence of tics related to exaggerated self-grooming (licking or chewing their paws); the display of aggressiveness.

We interpreted the ethograms according to data from Table 2. The resulting data were analysed statistically by computing an Average and Standard Deviation (SD) in Microsoft Excel. We also calculated the statistical significance of differences between the lots with the help of the t test (Student), also using Microsoft Excel.

RESULTS AND DISCUSSIONS

By analyzing the resulting ethograms within our experiment, we made the following observations:

- a 60% decrease in food intake for dogs from lot 1 when compared to those from lot 2;

Table 2

The effects of depression and stress on target behaviours for the experiment

Assessed behaviour	**Evolution under depression**	**Evolution under eustress**	**Evolution under distress**
Food intake	decrease	increase	decrease
Duration of sleep periods	increase	normal	decrease
Duration of self-grooming periods	increase	normal	decrease
Tics related to exaggerated self-grooming (licking or chewing of paws)	increase	absent	absent
Display of aggressiveness	increase	normal	increase

Table 3

Centralised table of ethological observations from the experiment

Assessed behaviour	**Lot 1**	**Lot 2**
Food intake (g)	250	400
Duration of sleep periods (%)	75%	60%
Duration of Self-grooming periods (%)	10%	5%
Tics related to exaggerated self-grooming (licking or chewing of paws) [no. of times these were observed]	6	-
Displays of aggressiveness (no. of times these were observed)	10	3

- a 20% increase in sleep periods for lot 1 dogs when compared to those from lot 2;
- a 50% increase in self-grooming behaviours for dogs from lot 1 when compared to those from lot 2;
- excessive self-grooming behaviours were only present in dogs from lot 1;
- a 70% increase in displays of aggressiveness for dogs from lot 1 when compared to those from lot 2.

By analysing these observations and using the assessed behaviours as indicators of a depressive state, we can conclude that animals from lot 1, that were constantly kept in their boxes and paddocks, manifest a higher number of behavioural patterns indicative of depression. No signs of depression were visible in animals from lot 2, which were doing activities specific to canine-assisted therapy and exposed to the associated stress stimuli. Moreover, the stress stimuli used in our experiment are reasonable in duration, nature and intensity, and as such induce eustress, also known as good stress. This, in turn, helps tone, strengthen and amplify the body's adaptive capabilities.

As for the haematological exams, for animals from lot 1, the number of red blood cells was 45% lower in the second sample as compared to the first sample. These differences are statistically insignificant (P>0.05). For lot 2, the number of red blood cells was 1.95% higher in the second sample as compared to the first sample. This represents a statistically significant difference (P<0.05). (see Table 4)

These results indicate a toning, positive effect on the erythrocytic population (polycythaemia) in the dogs involved in canine-assisted therapy. The differences we observed could be attributed to the specifics of the exercises that the animals perform, which imply moderate physical effort and the associated eustress, but also coming into contact and socializing with the patients. For dogs from lot 1, there were no eustress stimuli, as the animals didn't get any extra stimuli as compared to everyday life in the dog kennel. These results concur with those published by Riemer and Melco, who observed similar effects with the general adaptation syndrome in dogs. The results of the haematocrit and haemoglobin evaluation, in the conditions of our experiment, were in accordance with what we got from the evaluation of the number of red blood cells (Table 4). Therefore, we recorded the evolution of these parameters in close connection to the changes in the number of red blood cells.The results underline the fact that there's a more intense depressing potential for the benchmark lot, the one kept under normal conditions in the dog kennel from the treatment.

For lot 1, the number of white blood cells increased by 0.98% in the second sample as opposed to the first sample. These differences are statistically insignificant (P>0.05). For lot 2, the number of white blood cells was 17.54% higher in the second sample when compared to the first sample. These differences are statistically significant (P<0.05). (Table 5)

The proportion of neutrophils within the leukocyte formula for lot 1 was 0.51% lower in the second sample as opposed to the first sample. These differences are statistically insignificant (P>0.05). The ratio of neutrophils within the leukocyte formula for lot 2 increased by 5.49% in the second sample when compared to the first sample. These differences are statistically significant (P<0.05). The proportion of lymphocytes within the leukocyte formula for lot 1 was 1.63% higher in the second sample as opposed to the first sample. These differences are statistically insignificant (P>0.05). The proportion of lymphocytes within the leukocyte formula for lot 2 decreased by 12.65% in the second sample when compared to the first sample.

Table 4

The values of the hemogram for the two experimental lots

Lot	Average number of red blood cells (M/μL)		Average haematocrit (%)		Average haemoglobin (g/dl)	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1 Benchmark (n=5)	6.54±0.42	6.51±0.41	45.4±4.31	45.1±3.52	15.5±0.32	15.2±0.44
2 Experimental (n=5)	6.65±0.42	6.78±*0.33	45.5±3.83	47.7*±4.21	15.3±0.24	16.5*±0.45

Table 5

The values of the leukogram for the two experimental lots

Lot	White blood cells (K/μL) Average ±SD		Neutrophils (%) Average ±SD		Lymphocytes (%) Average ±SD	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1 (n=5)	10.2±0.40	10.3±0.32	78.2±2.25	77.8±2.22	30.5±1.52	31.0±1.62
2 (n=5)	11.4±0.22	13.4*±0.22	78.2±2.45	82.5*±1.53	31.5±1.82	27.5*±1.92

*P<0.05

These differences are statistically significant ($P< 0.05$). These results indicate a more intense eustress effect in dogs that were involved in canine-assisted therapy than in those that were kept in the dog kennel.

For lot 2, these results can be explained by the increase of the glucocorticoid hormones in the blood. These hormones induce the centralisation of neutrophils and inhibit their adherence to the vascular endothelium, thereby delaying diapedesis. This leads to the increase of the number of white blood cells. Not least, the increase of the glucocorticoid hormones in the blood leads to tissue sequestration of lymphocytes and reduce their ratio within the leukocyte formula. These results concur with those published by Riemer and Melco, who observed similar effects with the general adaptation syndrome in dogs.

Our results emphasise the fact that activities specific to canine-assisted therapy have an eustressing effect on participating dogs. It was clear that these eustress-inducing activities have a toning effect that improves behavioural and haematological parameters in the animals involved in the experiment.

CONCLUSIONS

The data from the resulting ethograms from our experiment indicated an increase in eustress-related behaviours in dogs used for therapy, but also how depression-specific behaviours evolve in dogs that are isolated in boxes and paddocks. Furthermore, we noticed that activities specific to canine-assisted therapy for children with disorders on the autism spectrum led to a significant increase of the haematocrit, haemoglobin and the number of red blood cells in dogs used for therapy, indicating a polycythaemia toning effect resulting from eustress. This sort of evolution was not seen in dogs that were isolated in boxes and paddocks.

Activities specific to canine-assisted therapy for children with disorders on the autism spectrum resulted in an increased number of white blood cells and neutrophils for dogs that were used for therapy. However, the ratio of lymphocytes in the blood of dogs used for therapy increased and led to a confirmation of eustress induced by the activities of therapy dogs.

Eustress generated by canine-assisted therapy activities had a positive effect on the overall mood, specific behaviours and the haematological profile in dogs that were used in the experiment.

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