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Serum levels of cortisol, adrenaline, noradrenaline, glucose and platelet counts in cats depending on the behavioural assessment of acute stress

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Summary

The aim of this study was to combine the estimated intensity of acute stress experienced by cats in the veterinary clinic with the assessment of serum cortisol, adrenaline, noradrenaline, glucose and platelet counts. The authors aimed to answer the question of whether the generally known acute stress markers correlate with the degree of stress experienced by cats in the veterinary clinic. Fifty clinically healthy cats were studied. Behavioural assessment of stress was performed according to the modified criteria indicated by Kessler and Turner under conditions of minimal stress recommended for veterinary medicine. Serum levels of cortisol, adrenaline, noradrenaline, glucose and platelet count were determined by recognized and commonly used methods. The cats examined in the clinic were divided into 3 groups according tothe type of stress reaction. In 8 (16%) cats, the behaviour was "relaxed", 25 (50%) cats showed "tense" stress reactions, and in 17 (34%) cats, strong reactions of fear were noted. Serum levels of cortisol, adrenaline, noradrenaline, glucose and platelet count differed between the groups, but the differences were not statistically significant and did not correlate with the degree of acute stress in cats. It is concluded that a large number of cats (84%) experience stress in the clinic and that the working methods of veterinary teams should be further improved to minimize it. When cats exhibit less fear during veterinary examinations, their owners are more likely to schedule examinations, and veterinarians are more likely to obtain accurate measures during the examination, which improves the overall welfare of the cat. Another conclusion is that the biochemical parameters tested did not meet the conditions to be treated as measures of acute stress in cats. Therefore, further research is needed to find indicators associated with the degree of acute stress in cats.

Keywords: acute stress, behavioural assessment, blood platelets, catecholamines, cortisol, glucose

Both cats and dogs undergo routine veterinary visits and examinations throughout their life. In recent years, there has been an increase in the awareness that certain forms of human behaviour in these situations may negatively affect the health and welfare of animals (1, 6, 9, 13, 14, 19). There has been, however, little research conducted into various forms of behaviour towards cats during these visits. The well-known sensitivity of cats andtheir stress reactions make veterinary visits technically difficult and have a negative impact on examination results. During veterinary visits, behavioural and physiological changes occur (12, 21, 31). Fearful or aggressive behaviour as well as hormones released in response to stress may influence the assessment of clinical pathology and make its interpretation complicated. Therefore it seems necessary to make efforts to reduce the intensity of stress during veterinary visits and to pay particular attention to the values of basic stress parameters related to the level of manifested stress in order to better interpret the results of clinical and laboratory examinations of cats (5, 8, 16, 17, 32, 35).

Guidelines on medical behaviour which are based on knowledge about the behaviour of cats have recently been published. Their objective is to increase the security of veterinarians, and at the same time to minimise the fear and pain of cats undergoing clinical examinations (2, 9, 18, 25, 26, 30, 33). Home visits have also been encouraged in order to avoid stress connected with transport and to reduce stress associated with the clinical environment (4, 21, 24). Although the impact of stress on physiological parameters and clinical pathology has been well researched in many animal species, there is little data concerning cats (12, 14). Reducing the level of stress during a medical examination may result in increased accuracy of the results, in an earlier detection of disease, in better general health of the cat and in greater comfort for the animal owners.

The behavioural assessment of the level of stress experienced by catsis based on observation. There are several protocols proposed for clinical purposes, e.g. the Kesslerand Turner scale (3, 7). They are all based on a subjective assessment of the body posture and its elements, movements and reactions of animals exposed to new, unknown stimuli. These protocols are not perfect, and their authors emphasize the need for their improvement, but they have the advantages ofbeing simple, easy and quicktouse under clinic conditions.

In view of the facts set out above, the aim of the study was to determine the level of stress of cats in veterinary offices on the basis of behavioural assessment and measurements of serum cortisol, adrenaline, noradrenaline, glucose and blood platelet counts. The results of these analyses may contribute to the debate on behavioural methods of stress assessment. They may also fill knowledge gaps concerning the impact of stress on the serum level of the selected stress indicators, glucose and blood platelets in cats,which makes it possible to better interpret the results of clinical and laboratory examinations.

Material and methods

Animals and behavioural assessment. The study was carried out on 50 client-owned cats (30 females and 20 males) aged 8 months to 16 years, clinically healthy or with minor illnesses such as conjunctivitis, irritation of the external ear canal and mild skin lesions of small size. The study was carried out at the Department and Clinic of Animal Internal Diseases, Faculty of Veterinary Medicine in Lublin.

The owners usually waited in an area of the general waiting room reserved for cat owners, with a room screen separating it from the reception/general waiting area. The length of time in the waiting room was shortened to a minimum because patients were invited to the office immediately after registering.

The cats were gently lifted from the carrier by the owners or by the veterinarian in a few cases, usually the owners waited until their cat came out on its own. The cats were treated with empathy by both the owners and veterinarians.

The cats underwent clinical control examinations together with blood collection. They were assessed between 2 and 4 on a 5-level BCS scale. The haematological and biochemical parameters of the cats were within normal physiological limits. The results of FIV and FeLV tests were in each case negative.

The behavioural responses of the cats to the veterinary visit werealways assessed in the same way. The assessment was performed by the same doctor (K.W.), who observed the cats within the first 10-15 minutes of the visit. The visit was prepared and proceeded according to recommenda-

Tab. 1. Criteria and results of behavioral assessment of cats during a visit to a veterinary clinic. Modified system based on Kessler criteria (7)

	Score		
	1. Fully and weakly relaxed	2. Weakly and very tense	3. Fearful and very fearful
Body	Laid out on side or on back; i: laid ventrally or half on side or sitting; a: standing or moving, back horizontal	i: laid ventrally, rolled or sitting; a: standing or moving, body behind lower than in front	i: laid ventrally, sitting or crouched directly on top of all paws, may be shaking a: standing or moving, body behind lower than in front or whole body near to ground, crawling, may be shaking
Stomach	Exposed or not, slow or normal ventilation	Not exposed, normal ventilation	Not exposed, normal or fast ventilation
Legs	Fully extended	i: bent a: when standing, hind legs bent in front extended or bent near to surface	i: bent a: bent near to surface
Tail	i: extended or loosely wrapped a: up or loosely downwards	May be twitching i: on the body, curved backwards or close to the body a: up or tense downwards or curled forward, may be twitching	i: close to the body a: curled forward close to the body
Head	Laid on surface or over the body, some movement	Over the body or pressed to body, little or no movement	On the plane of the body, near to surface or lower than body, less or no movement
Eyes	Closed, half or normal opened, may be blinking slowly	Normal, widely open or pressed together	Widely or fully opened
Pupils	Normal	Normal or partially dilated	Dilated or fully dilated
Ears	Half-back or erected to front or back and forward on head	Half-back or erected to front or back and forward on head	Partially or flattened, possible back on head
Whiskers	Lateral or forward	Lateral or forward	Lateral, forward or back
Vocal	None	Meow, plaintive meow or quiet	Plaintive meow, yowling, growling or quiet
Activity	Sleeping, resting, alert or active, may be playing	Cramped sleeping, resting or alert may be actively exploring, trying to escape	Alert, may be actively trying to escape or prowling; motionless

Explanations: i – cat is inactive; a – cat is active tions designed to minimise stress (9, 24, 32). The behavioural assessment was performed on the basis of a modified Kessler and Turner categorisation (7). The authors modified it by reducing the 7 levels of the scale to 3 levels (Tab. 1). This modification made it possible to distinguish 3 groups of cats. The first group was made up of the so-called "fully and moderately relaxed cats (1 and 2 on the Kessler and Turner scale), the second group of "moderately tense and very tense" cats (3 and 4 on the Kessler and Turner scale) and the third group of "fearful and very fearful" cats (5, 6 and 7 on the Kessler and Turner scale).

Laboratory testing and statistical analysis. Blood samples were collected from the jugular vein into 2 ml syringes with a BD Vacutainer system into K2-EDTA tubes for haematological tests and plain tubes for serum analyses. The blood was always collected between 10:00 a.m. and 12:30 p.m. because of the diurnal variation in hormone levels.

The concentration of cortisol was determined with of an IMMULITE 1000 apparatus, Siemens (Germany). Enzyme Conjugate – Cortisol conjugated to peroxidise from horseradish. The inter-assay CVs ranged between 6.5% and 7.7%. The limit of quantification was 6.9 nmol/l. Epinephrine and norepinephrine levels in plasma were determined by a specific enzyme test 2-cat (A-N) Research ELISA, LDN, Germany. Sensitivity was 6.6 pg/ml for epinephrine and 2.6 pg/ml for norepinephrine.

The hormonal assays for epinephrine and norepinephrine were carried out using an ELISA Microplate Reader (Molecular Devices Spectra Max M2). The blood glucose level was quantified by the hexokinase enzymatic method with an ABX Pentra 400 (Horiba Medical, Poland). The platelet count was determined with a 3-diffscil Vet abc Plus+ haematology analyser and verified by assessment of a blood smear.

The statistical analysis of the results was performed with the Mann-Whitney test for non-parametrical data under examination. Differences were considered statistically significant at $P < 0.05$. The distribution of data was analysed by the Shapiro-Wilk test.

Results and discussion

The behavioural assessment of the cats was performed on the basis of criteria set out in table 1. The analysis of their reactions in a veterinary office made it possible to observedifferent types of behaviour. As many as 8 animals (16%) were classified in the group of "relaxed" cats, 25 animals (50%) were classified in the group of "tense" cats, and 17 (34%) were categorised as "scared" cats.

The results for the serum concentration of cortisol, adrenaline, noradrenaline, glucose level and blood platelet count in the three groups of cats are presented in table 2.

The concentration of serum cortisol was lowest in the group of "relaxed" cats and amounted to 3.9 ug/dL. The highest concentration, amounting to 4.7 ug/dL, was detected in the group of "tense" cats, and it was only slightly lower in the group of "scared" cats: 4.5 ug/dL. However, the differences between the groups were not statistically significant.

The concentration of adrenaline in the cats from group I varied considerably from one animal to another, and the atypical distribution of values made it impossible to usethem in the analysis. In the cats from group II, the concentration of adrenaline was 0.046 ng/mL, and in group III it was slightly higher: 0.052 ng/mL. No statistically significant differences were observed between the results for these two groups of cats.

The concentration of noradrenaline was 0.10 ng/mLin group I, 0.17 ng/mLin group II and 0.22 ng/mLin group III. The analysis of the noradrenaline trend revealed that its level in serum grew proportionally to the response to stress. However, statistical analysis demonstrated no statistically significant differences between the groups.

The concentration of glucose was lowest in the cats from group I: 106.7 mg/dL and highest in the cats from group II: 124.0 mg/dL. In the cats from group III, it amounted to 114.2 mg/dL. No increase in glucose concentration was observed with the increase in behavioural response to stress. In group II of the "tense" cats, the values of glucose were characterised by high standard deviations compared to those in group III. The statistical analysis demonstrated no statistically significant differences between the groups.

The blood platelet count was lowest in the cats from group I, amounting to 318,200, and highest in group II: 435,800. In group I, the platelet count was 351,300. The statistical analysis of the results revealed no trend and demonstrated no significant differences between the groups.

The behavioural assessment of cats under conditions of acute stress led to their categorisation into 3 groups. The behaviour assessed as "relaxed" occurred in only 8 animals, which represented merely 16% of the sample. What all of them had in common was that their owners hadkept them since the cats were 4 to 8 weeks old and that they got on well with other animals and humans. The owners also stressed the fact that their cats were gentle and friendly. There is no doubt that all these facts contributed to the trusting approach of the animals to new stimuli,which largely explains their calm behaviourin veterinary offices.

The reactions of animals assessed as "tense" were found in 50% of the cats. As much as 34% of cats were

Tab. 2. The serum concentration of cortisol, adrenaline, noradrenaline, glucose level and blood platelets count in examined cats

Number of cats (n = 50)	Cortisol (mg/dl)	Adrenaline (ng/ml)	Noradrenaline (ng/ml)	Glucose (mg/dl)	Platelets ($10^3/mm^3$)
1. Fully and weakly relaxed (n = 8)	3.9 ± 2.38	individual variability	0.10 ± 0.10	106.7 ± 37	318.2 ± 147
2. Weakly tense and very tense (n = 25)	4.7 ± 2.58	0.046 ± 0.02	0.17 ± 0.12	124.0 ± 93	435.8 ± 198
3. Fearful and very fearful (n = 17)	4.5 ± 2.97	0.052 ± 0.03	0.22 ± 0.20	114.2 ± 48	351.3 ± 106

assessed as "scared". A total of 84% of the cats observed in the office manifested moderate to high degrees of stress. These observations are in line with reports from other authors (1, 10). They emphasise and justify the need for a continuous effort to minimise stress as part of clinical activities leading to a diagnosis of disease.

The second objective of the study was to evaluate the correlation between behavioural changes in cats and the selected parameters, which could indicate the level of stress experienced by cats underveterinary office conditions. Clinicians aware of such a correlation could more accuratelyinterpret physical and laboratory test results. However, the results of the study have notrevealedsignificant differences and correlations between stress behaviours in cats and their levels of cortisol, adrenaline, noradrenaline, glucoseand blood platelets. It seems very unlikely, however, that the inability to link cortisol and the other parameters with behavioural changes resulted from the lack of correlation. It can be speculated that the apparent lack of correlationwas due mainly to the disruption of the behaviour of the cats and insufficient precision in identifying their behaviours.

The Cat Stress Score (CSS) developed by Kessler and Turner is a commonly used method of behavioural stress assessment (9, 24, 32). Despite the universality of its application, several users and the authors themselves suggest the need for further improvement (3, 7). The assessment is subjective, static and based on behaviours presented in short, 1-minute time intervals. It appears that the CSS may be affected by various factors, such as breed, age, sex, castration, individual experience of cats, frequency of veterinary visits, etc. Since the CSS may be so sensitive to the measured parameters, one has to be particularly careful in interpreting the results based only on the CSS scale as a method to measure stress.

The measurements of serous or plasmatic levels of cortisol have the advantage of reflecting temporary concentrations in blood, which is considered an accurate measurement of active levels in the organism. The cortisol level in the blood increases several minutes after the occurrence of a stressor. This has been demonstrated in cats between the 5th and the 15th minute. This rapid change makes it possible to measure a short-term stress, for example, during a veterinary visit and an examination. A disadvantage of this type of examination is the fact that the procedure is invasive and may affect the results (27). Our results do not reveal differences between the groups of cats evaluated by the CSS, which is consistent with the findings of other authors (11, 15). Nibblett et al., 2015 concluded no differences even between serous cortisol levels in cats tested at home and under clinic conditions (15). McCobb et al. found no correlations between the results of the CSS and the ratio of cortisol to creatinine in urine (11). Moreover, no correlation was found between the CSS and cortisol metabolites in faeces (27). Interestingly, Tanaka et al. found a correlation of a higher CSS with the development of upper respiratory diseases and reduced appetite in cats (29).

The stress hormones include adrenaline and noradrenaline. The rate of their secretion and their serous levelsare used for assessment of stress in both humans and animals. The serous level of adrenaline and noradrenaline found in our research did not differ significantly between the groups of cats, but the results for both hormones showed a linear relationship with a marked trend for noradrenaline, which seems to confirm the assumption that behavioural stress increases the level of noradrenaline. It is difficult to compare these results with others due to the lack of similar arrangements in the assessment of the acute stress model in cats.

The elevated level of glucose in blood occurs in animals in the course of stress because of hormones, including cortisol and adrenaline, which oppose the action of insulin. Scientific reports confirm the impact of catecholamine in the development of stress hyperglycaemia in humans and animals. Studies of cats also confirmed this hypothesis (4, 23). The elevated level(s) of glucose/catecholamine/glucose and catecholamine was/were demonstrated in a veterinary office compared to tests performed at home (15). It was suggested that the concentration of catecholamine may be a valuable indicator in the assessment of cats with hyperglycaemia. The catecholamine concentration rapidly changing in response to a weak stimulus can explain the large variation in the level of catecholamine in the cats involved in the present study. A study carried out by Rand on cats with transient hyperglycaemia demonstrated that when it is associated with a disease, it occurs more often (3.2%) than when it is associated with diabetes (0.57%) (23). When hyperglycaemia overlaps with the one associated with diabetes during stress connected with the blood collection, it cannot be ruled out that the level of glucose will increase.

As expected, in this study, the cats remaining "tense" presented higher values of glucose than the "relaxed" cats, although the differences were not statistically significant. It should be noted that glucose levels showed a large individual variation, particularly in the group of "tense"cats. There have been reports of stress hyperglycaemia in cats with the levels amounting to 360-613 mg/dL (22, 23), but in the present study, the level of glucose in blood exceeded 200 mg/dL only in two cats (1 cat from group B: 315.9 mg/dL and 1 cat from group C: 253.3 mg/dL). Similar results were obtained by Rand et al. in a study where glucose peakedat 285 mg/dL (23). Plotnick et al. report that an increase in the level of glucose in blood from 288 mg/dL to 396 mg/dL occurred in cats who were stressed by a veterinary visit (20).

The clinical significance of in-office glycaemia consists in the difficulty of differentiating whether it is physiological or pathological. This underlines the importance of the need to reduce stress in cats during veterinary visits and blood collection in order to minimise the risk of misinterpretation and maximise the reliability of examinations.

Another familiar correlation is the fact that stress increases the activity of the sympathetic system and increases the output of pro-inflammatory cytokines and catecholamine. Adrenaline and noradrenaline intensify the aggregation of blood platelets, which may obstruct vascular blood flow or even block it. In the present study, the blood platelet count increased in the group of "tense" cats, exceeding the number recognised as physiological, although the differences between the groups proved to be statistically insignificant. Due to the wide individual variability and atypical distribution of adrenaline values in the "relaxed" cats, the authors did not analyse its impact on the blood platelet count in the cats.

The techniques ofdealing with stress in cats and reducing stress during a medical examinationproved effective. Despite the significant number of animals demonstrating stress (84%), it was possible to complete all physical examinations with a minimum degree of difficulty, without any injury to the owners or staff. However, there is still room for improving the efficiency of medical examinations while minimising stress in cats, e.g. by using headphones while measuring arterial blood pressure by the Doppler method or avoiding injections of cold fluids into veins. This may encourage more frequent veterinary visits and increase the accuracy of examinations performed on cats.

The attempt to relate stress behaviours of cats to the generally known stress indicators in blood serum did not produce clear results. None of the test parameters can be a sufficient indicator of the stress level in cats, although the trend line is best marked by the serous level of noradrenaline. The data from the measurement of cortisol and catecholamine varied widely from one individual to another and showed an atypical distribution. One conclusion from this study is the need for further research indicators that may prove specific for strong stress reactions in cats. Recently, the potential of metanephrine (ME) and normetanephrine (NME) in the plasma and urine of catsas markers of stress response has been investigated, but preliminary results of those studies have not led to a breakthrough (28, 34).

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