

# Correlation between motor laterality, temperament and cortisol levels in dogs

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© ANETA STRACHECKA<sup>2</sup>, © PATRYCJA STANISZEWSKA<sup>2</sup>

<sup>1</sup>Department of Animal Ethology and Wildlife Management, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

<sup>2</sup>Department of Invertebrate Ecophysiology and Experimental Biology, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

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Garbiec A., Karpiński M., Wojtaś J., Strachecka A., Staniszevska P.

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### Summary

This study aimed to evaluate the correlation between motor lateralisation, temperament, and serum levels of cortisol. The study group consisted of 39 clinically healthy dogs: 21 females and 18 males. Lateralisation and temperament were assessed during periodic animal health checks using a standardised tape test and a proprietary assessment algorithm. The median cortisol level in the dogs was 4.6 ng/ml. The median cortisol level was 4.31 ng/ml in dogs under 3 years of age and 4.92 ng/ml in dogs over 3 years of age. The median cortisol level was 4.21 ng/ml in left-pawed dogs and 4.8 ng/ml in right-pawed dogs. Statistically significant differences were found between cortisol levels and temperament. The median cortisol level was 5.06 ng/ml in extrovert dogs and 4 ng/ml in introvert dogs.

**Keywords:** dog, cortisol, lateralisation, temperament

Many researchers have long studied motor lateralisation to determine the relationship between an animal's laterality and independent variables examining the relationship between laterality and the sex, age, and maintenance of tested animals (18, 24). In the above studies, it was observed that males tend to use the left paw while females tend to use the right, with increased activity of the right hemisphere in responding to novelty, and the fact that older dogs also showed a stronger preference for the right paw than younger dogs. Schneider et al. (30) assessed the relationship between laterality and temperament; however, found no evidence of a relationship between paw preference and temperament in their study. Foyer et al. (9) studied the relationship between stress levels and motor lateralisation in working dogs and showed a high positive correlation. Quaranta et al. (25) investigated and demonstrated the relationship between stress and reduced immunity levels in dogs. Wells et al. (37) showed no significant results between motor lateralisation and the adaptive abilities of dogs. However, the Wells (35) study found that the paw preference test is helpful in assessing the emotional functioning and mental well-being of dogs and cats. A study by Wells et al. (36) provided little evidence to support an association

between paw preference and behavioural problems in the domestic dog. Motor lateralisation in dogs can be determined by assessment of the dominance of one of the front paws in a sticky tape test placed on the dorsal part of the muzzle (3, 4, 9, 27).

Stress is a body's biological response triggered by extraordinary circumstances defined as stressors threatening its natural homeostasis (7). Activation of the HPA pathway by a stimulus leads to an increase in circulating glucocorticoids in the blood. The most important indicators of stress severity levels in mammals are cortisol and corticosterone (34). The assessment of cortisol levels is an effective method for determining acute and chronic stress levels in dogs in various biological materials (5). Determining the severity of the stress response triggered by various stimuli is becoming increasingly popular as a helpful tool in assessing animal welfare. Furthermore, it provides an opportunity to implement optimal changes in space management in veterinary clinics (38).

In addition, it has been proven that lateralisation is associated with the involvement of the cerebral hemispheres in emotional functioning. The study of bias in animals has become crucial in recent years due to the understanding of the emotional functioning of the brain

(12). Functional cerebral asymmetry causes changes in animal behaviour that can be objectively measured by non-invasive and practical methods, including personality and temperament assessments (1, 27, 28).

In the ethological literature, there are two essential terms, i.e. temperament and personality, where temperament is an integral part of the personality (16). Taking advantage of the fact that caretakers know their animals best, having observed them in different situations throughout their lives, researchers can provide key information to help estimate the animal's temperament by answering simple questions (17).

This study was designed to demonstrate the correlation between the severity of the stress response in dogs during veterinary activities and the motor laterality and temperament of the animals studied. The authors wanted to check that innate tendencies, i.e. asymmetry of brain hemispheres or temperament, can influence the stress reaction intensity during a visit to the veterinary practice. Serum cortisol level was used to indicate the acute stress response severity. Motor lateralisation was determined using a standardised test with the adhesive tape placed on the dorsal side of the dog's muzzle. Temperament was determined upon completing a self-administered questionnaire by the animal's handler (17).

Although there are many reports on the association between lateralisation and cortisol levels or lateralisation and behaviour, we did not find many reports linking cortisol levels, motor lateralisation and temperament.

## Material and methods

**Animals.** The study group consisted of 39 dogs (*Canis familiaris*) (21 females and 18 males) ranging in age from 1 to 6 years. All the animals came from a shelter for homeless animals or an animal help organisation. All animals were crossbred. The animals were divided into two age groups: under 3 years old (23 dogs) and over 3 years old (16 dogs). There were 21 female dogs and 18 male dogs in the study group, and 37 dogs were neutered. 13 individuals showed a left-sided preference, while 26 dogs were right-pawed. Extrovert temperament was found in 20 dogs, and 19 dogs exhibited introvert traits. The animals were clinically healthy, were not treated for cancer or metabolic diseases, had valid vaccinations against infectious diseases, and were regularly dewormed. Based on the interview with the temporary handlers, none of the dogs exhibited extreme behaviours such as anxiety or aggression. Animals had no restricted access to food and drank prior to testing. They were invited to the veterinary practice for a routine periodic health examination, including a complete clinical examination and laboratory tests. The clinical examination lasted approximately 30 minutes; at the end, blood was drawn for analysis. The laboratory test indicators showed no abnormalities. Morphological indices, i.e. hematocrit, haemoglobin, erythrocytes, leukocytes, and platelets, were within the range of reference standards for the species. Biochemical indicators, i.e. alkaline phosphatase, alanine

aminotransferase, aspartate aminotransferase, creatinine, urea, cholesterol, and glucose, were within reference standards for the species.

**Test procedure.** All methods used in the study complied with the Polish Law of January 15, 2015, on the Protection of Animals Used for Scientific or Educational Purposes (Journal of Laws 2015, item 266) and the Law of December 18, 2003, on Animal Treatment Facilities (Journal of Laws 2019, item 24). Biological material (blood) was collected for routine laboratory testing of the study animals, and the pawing and temperament assessments were performed with the full consent of the animal caretakers. Cortisol levels were determined once in parallel with the other parameters. They were compared with reference values for the species.

**Paw preference assessment.** The motor lateralisation was assessed by observing which paw the dog used to remove the piece of adhesive tape from the bridge of its nose: the same as in the previous study Garbiec et al. (10). The first three repetitions were performed with the participation of a carer in the veterinary office. The animal's guardian performed the remaining repetitions at the place where the dog was staying (within a week). The lateralisation was calculated using the formula  $z = (R - 0.5N) / \sqrt{(0.25N)}$ , where R signifies the number of R paw uses and N signifies the sum of L plus R paw uses. Dogs with mean  $z \geq 1.96$  were classified as right-pawed, and dogs with mean  $z \leq -1.96$  were classified as left-pawed. For each tested animal, the HI (handedness index) parameter was also calculated: it was 1.0 for dogs with the left-sided preference and -1.0 for dogs with the right-sided preference (33). Due to the small number of bilateral dogs or the doubt of the result, these dogs were not included in the study.

**Temperament assessment.** Temperament was assessed using a self-administered questionnaire with 24 selected temperament traits (Tab. 1). The dog handlers voluntarily completed the questionnaire. Temperament was assessed using a logistic regression model by Karpiński et al. 2022. For each dog, the number of extrovert traits (ET) and introvert traits (IT) were counted from the questionnaire. The dogs were classified as extroverts or introverts for the probability values of  $p > 0.5$  and  $p < 0.5$ , respectively.

**Tab. 1. Authors' questionnaire with the 24 selected temperament traits**

Yes	Trait	No
	Wise	
	Sociable	
	Curious	
	Cheerful	
	Dominant	
	Noisy	
	Disobedient	
	Hyperactive	
	Clever	
	Impatient	
	Bold	
	Territorial	
	Stubborn	
	Loner	
	Aggressive	
	Lazy	
	Greedy	
	Alert	
	Unpredictable	
	Fearful	
	Distrustful	
	Insecure	
	Absent-minded	
	Timid	

Based on statistical analyses, two clusters of selected features were obtained. Cluster 1 comprised the following traits: wise, cheerful, impatient, bold, sociable, curious, noisy, hyperactive, territorial, clever, dominant, and disobedient. In turn, cluster 2 comprised the following traits: stubborn, lazy, greedy, loner, timid, fearful, unpredictable, distrustful, insecure, aggressive, absent-minded, and alert. Traits from cluster 1 were assigned to individuals with an extroverted temperament, while the traits from cluster 2 are typical of introverted animals (17).

**Laboratory analysis.** The cortisol concentrations in the samples were determined with the DRG Cortisol HS ELISA assay. The procedures followed the manufacturer's instructions. The cortisol concentrations were expressed in ng/ml.

**Statistical analysis.** The obtained results were subjected to detailed statistical analysis. The values of the analysed quantitative variables are presented as the median value, and the qualitative variables as shown as the number and percentage. For qualitative characteristics, the Chi<sup>2</sup> test with Yates correction was used to detect the existence of correlations between the analysed variables. The normality of the distribution of the variables in the study groups was checked using the Shapiro-Wilk normality test. The Student's t-test was used to examine the differences between the two groups, and if the conditions for its use were not met, the Mann-Whitney U test was used. The r-Pearson correlation was used to assess the linear relationship between the age and the analysed variables. A significance level of  $p < 0.05$  was adopted to indicate the existence of statistically significant differences or relationships. The database and statistical tests were performed using Statistica 9.1 computer software (StatSoft, Poland).

## Results and discussion

The median cortisol level was 4.31 ng/ml in the dogs under 3 years of age and 4.92 ng/ml in the dogs over 3 years of age. There were no significant statistical differences in the cortisol levels between the two groups (Tab. 2).

**Tab. 2. Comparison of medians for cortisol in dogs under 3 years of age and over 3 years of age**

Analysed variable	Median levels of cortisol in the dogs under 3 years	Median levels of cortisol in the dogs over 3 years	P-value	Correlation analysis
Cortisol [ng/ml]	4.31	4.92	0.161	0.48

**Tab. 3. Comparison of medians for cortisol in L-paws and R-paws dogs**

Analysed variable	Median levels of cortisol in L-paws dogs	Median levels of cortisol in R-paws dogs	P-value
Cortisol [ng/ml]	4.21	4.79	0.78

**Tab. 4. Comparison of medians for cortisol in extravert and introvert dogs**

Analysed variable	Median levels of cortisol in extraverted dogs	Median levels of cortisol in introverted dogs	P-value
Cortisol [ng/ml]	5.06	4.00	< 0.001

A detailed comparison of the individual analysed variables (cortisol) was also made in the extroverted and introverted dogs as well as left and right paws. The cortisol levels were not significantly different between the left- and right-pawed dogs. The median cortisol level was 4.21 ng/ml for the left-pawed dogs and 4.8 ng/ml for the right-pawed dogs. No statistically significant differences were also obtained between the motor lateralization (Tab. 3).

The median level of cortisol was 5.1 ng/ml in the extrovert group (E) and 4 ng/ml in the introvert group (I), which was a statistically significant result (Tab. 4).

No statistically significant differences in the cortisol or motor lateralisation and temperament between the male and female sexes or between the neutered and unneutered dogs (Chi<sup>2</sup> test:  $0.14 < P < 0.95$ ).

A visit to the veterinary practice is considered a highly stressful situation for companion animals. In the present study, we determined cortisol levels as a well-known indicator of the stress response severity and their correlation with motor lateralisation and temperament (40). The results obtained showed that the cortisol levels in the studied group of dogs were higher than the reference values in dogs reported by Hennessy et al. (14), Meyer and Harvey (19), or Muller et al. (21), i.e. 0.1-0.6 ng/ml. The median cortisol level in the dogs analysed in the present study was 4.6 ng/ml, significantly higher than average values. Similar conclusions were reached in 2012 by Hekman et al. (13), who recorded a mean cortisol level of 8.6 ng/ml in dogs admitted for surgery. Veterinary activities are undoubtedly a significant emotional challenge for animals; however, there were no significant differences in cortisol levels between male and female dogs. Similar to the studies of Ocklenburg et al. (22), there was no significant difference between sex and laterality in dogs, with female cats more likely to be right-sided than males.

However, it should be taken into account that the measurement of cortisol has some limitations. Its level depends on many factors, including the time of day when the test takes place, individual variability and external stressors, but it is still the most common marker for assessing the severity of the stress reaction (35).

Motor lateralization is widely studied in dogs, and scientists have proven that sex and age significantly impact paw preferences, e.g. older dogs showed a stronger preference for the right paw than younger dogs (18). There is evidence that lateralization is deeply related to cognition in dogs. Understanding this relationship can help improve dogs' well-being and relationships with humans. Thanks to non-invasive lateralization assessment techniques, we can assess dogs' cognitive abilities and emotional states and improve everyday veterinary and behavioural practice (31). Studies conducted on other primate species show behavioural



differences between left-handed and right-handed adult marmosets, i.e. different reactions to new stimuli or social behaviour (26). Paw preference may improve cognitive abilities (15).

Researchers studying the correlation between stress response and lateralisation have demonstrated a trend toward higher cortisol levels in animals with a left-sided paw preference (27) or more excellent behavioural response to stress (6). However, we found no significant differences between cortisol levels and motor lateralisation, the same as Batt et al. (3) and Barnard et al. (1), who showed there was little evidence of a relationship between motor lateralisation and cortisol activity. In their research, Salgirli Demirbas et al. (29) found that acute stress during motor lateralization tests may affect or interfere with the correct interpretation of the result. They found that in the group of dogs with higher cortisol levels, dogs more often did not show a specific paw preference (ambilateral). Previous studies in a hotel for dogs have shown that females tend to have higher levels of cortisol measured in saliva than dogs and that smaller breeds are characterized by a higher intensity of the stress response (39).

When considering the relationship between the age of the animals studied and the cortisol levels, no statistically significant differences were found, as in the study conducted by Mongillo et al. (20). In contrast, a study on 11 beagle dogs by Goy-Thollot et al. (11) showed that plasma cortisol levels increased with age. Similarly, when cortisol levels were compared between females and males, there were no statistically significant differences, as in the studies reported by Foyer et al. in (9) or Mongillo et al. (20).

The study showed a relationship between a dog's temperament and stress response. Animals with hyperactive status in the C-BARQ test also showed higher serum cortisol levels than calm animals (8, 9). Similarly, a study conducted by Hennessy et al. (14) demonstrated that pups that were bolder and more receptive to people showed higher cortisol levels on subsequent days in the shelter. Siniscalchi et al. (32) presented a comprehensive review of asymmetries for sensory and motor functions related to the emotional functioning of pets. In the work of Bernard et al. (2), a relationship between canine personality and lateralisation was observed, which concerned traits such as aggressiveness, fearfulness and sociability in dogs with specific laterality and bilateral. However, in the extensive Plueckhahn (23) report, no significant relationship was observed between paw preference and temperament in the study group of animals. In his study, the author emphasises that the assessment of a dog's temperament is very subjective in the opinion of its guardian.

It can be speculated that the discrepancies in the results obtained by many authors may be related to the different numbers of animals studied, the different

ways of maintenance, species differences, and differences in the temperament of the dogs.

Based on the results obtained, it can be concluded that there is no positive correlation between the animal's sex and the intensity of the stress reaction, temperament or brain asymmetry. Nevertheless, interesting results were obtained regarding the dog's temperament compared to cortisol levels. It can be concluded that dogs with extroverted traits are more susceptible to stress. It is therefore worth emphasizing that the assessment of dogs' innate tendencies has a significant impact on their behavior, as well as on how they cope in stressful situations; e.g. the dog shelter mentioned in the article, but also a hotel or a veterinary office. Knowing the paw's preferences and temperament is a helpful tool when working with a dog, socially interacting, and choosing appropriate grooming methods.

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**Corresponding author: Miroslaw Karpiński, DVM, PhD, Akademicka 13, 20-950 Lublin, Poland; e-mail: miroslaw.karpinski@up.lublin.pl**