UNIWERSYTET PRZYRODNICZY W LUBLINIE

WYDZIAŁ NAUK O ZWIERZĘTACH I BIOGOSPODARKI

Dyscyplina Zootechnika i Rybactwo

Lek. wet. Aleksandra Garbiec

Ocena zależności pomiędzy poziomem stresu a lateralizacją motoryczną i temperamentem u psów towarzyszących

Assessing the relationship between stress levels and motor lateralization and temperament in companion dogs

Praca doktorska

Doctoral thesis

Lublin, 2023

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Promotor:
Dr hab. lek. wet. Mirosław Karpiński, profesor uczelni
Uniwersytet Przyrodniczy w Lublinie

Lublin, 2023

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Panu dr hab. lek. wet. Mirosławowi Karpińskiemu, profesorowi uczelni
za okazaną pomoc, życzliwość oraz wyrozumiałość,
na każdym etapie mojej dotychczasowej ścieżki naukowej,
której owocem jest niniejsza praca doktorska.

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1. Wojtaś Justyna, Karpiński Mirosław, Czyżowski Piotr, **Garbiec Aleksandra**. Dogs' Stay in a Pet Hotel–Salivary Cortisol Level and Adaptation to New Conditions. Journal of Applied Animal Welfare Science, 2020, 1-6. DOI: 10.1080/10888705.2020.1781631.

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2. **Garbiec Aleksandra**., Karpiński Mirosław, Wojtaś Justyna, Czyżowski Piotr. The Relationship Between the Motor Lateralization and the Concentration of Cortisol and Tyrosine in Dogs Exposed to Stress During Veterinary Activities. Journal of Applied Animal Welfare Science, 2020 1-10. DOI 10.1080/10888705.2022.2082249

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3. Karpiński Mirosław, Wojtaś Justyna, **Garbiec Aleksandra**. Temperament Assessment Algorithm in Dogs. Animals. 2022, 12(5), 634. DOI 10.3390/ani12050634.

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Streszczenie

W czasach stale rosnącej empatii do zwierząt towarzyszących człowiekowi, ważnym aspektem wzajemnej egzystencji ludzi i psów stał się poziom nasienia reakcji stresowej, a tym samym dążenie do zapewnienia zwierzęciu odpowiedniego poziomu dobrostanu. Z roku na rok można zauważyć pozytywny trend nie tylko wśród opiekunów zwierząt, ale także osób pracujących na co dzień z czworonogami, aby zapewnić optymalne warunki z jednocześnie najniższym stopniem narażenia na bodziec stresowy. Idąc za tropem minimalizowania stresu u czworonożnych przyjaciół przeprowadzono badania czy wrodzone skłonności takie jak asymetria mózgu oraz temperament mają wpływ na poziom kortyzolu u psów w odpowiedzi na stresor.

Badania przeprowadzono na psach narażonych na czynniki stresowe tj. pobyt w hotelu dla zwierząt, pobyt w szpitalu dla zwierząt w oczekiwaniu na zabieg chirurgiczny kastracji oraz rutynowe czynności lekarsko – weterynaryjne obejmujące badanie kliniczne oraz pobranie materiału biologicznego (krwi) do kontrolnych badań laboratoryjnych. Badania zostały przeprowadzone w dwóch turach w latach 2019-2021 łącznie na 76 zdrowych klinicznie psach. Ponadto do opracowania algorytmu oceny psiego temperamentu w 2022 roku w badaniu wzięło udział 46 psów oraz ich opiekunowie (behawioryści zwierząt). Podczas badań oceniano lateralizację motoryczną za pomocą ustandaryzowanego testu z taśmą przylepną umieszczaną na grzbiecie nosa psa oraz poziom nasilenia odpowiedzi na stres na podstawie poziomu kortyzolu. Temperament jako wrodzoną część osobowości określano z wykorzystaniem algorytmu na podstawie przeprowadzonych ankiet z opiekunami psów. Analizowano takie wskaźniki jak prawołapność i lewołapność, poziom kortyzolu w ślinie oraz surowicy, rodzaj temperamentu z podziałem na ekstrawertyczny i introwertyczny.

Uzyskane wyniki badań pokazują, że zwierzęta podobnie jak i ludzie wykazują odmienną, osobniczą reakcje na stres. Badania przeprowadzone w hotelu ukazują tendencję do adaptacji tj. mediana poziomu kortyzolu w ślinie każdego dnia była niższa, co więcej mediana poziomu kortyzolu była niższa u samców niż samic, co może sugerować odmienną odporność na stres ze względu na płeć. Analiza zależności pomiędzy lateralizacją ruchową a stresem prowadzi do wniosku, że psy preferujące lewą łapę były bardziej podatne na bodziec stresowy, ponieważ poziom kortyzolu w surowicy tych osobników był znacząco wyższy. Dalsza część z przeprowadzonych badań nieuwzględniona w niniejszej pracy doktorskiej ujawniła, że psy o cechach ekstrawertycznych są bardziej podatne na stres niż psy introwertyczne.

Słowa kluczowe: pies domowy, stres, lateralizacja motoryczna, temperament

Summary

In these times of ever-increasing empathy for companion animals, an essential aspect of the mutual existence of humans and dogs has become the intensity level of the stress response and thus the desire to provide an appropriate level of welfare for the animal. Year after year, a positive trend can be observed not only among animal caretakers but also among people who work with quadrupeds on a daily basis to provide optimal conditions with, at the same time, the lowest level of exposure to the stress stimulus. Following the lead of minimizing stress in four-legged friends, studies have been conducted on whether innate tendencies such as brain asymmetry and temperament affect cortisol levels in dogs in response to a stressor.

The study was conducted on dogs exposed to stress factors i.e. staying in an animal hotel, staying in an animal hospital awaiting neuter surgery and routine veterinary activity, including clinical examination and collection of biological material (blood) for control laboratory tests. The study was conducted in three rounds between 2019 and 2021 on a total of 76 clinically healthy dogs. In addition, to develop an algorithm for assessing canine temperament in 2022, 46 dogs and their handlers (animal behaviourists) participated in the study. During the study, motor lateralization was assessed using a standardized sticky tape test placed on the back of the dog's nose and the severity of the stress response based on cortisol levels. Temperament as an innate part of personality was determined using an algorithm based on surveys with dog handlers. Indicators such as right-handedness and left-handedness, salivary and serum cortisol levels, and type of temperament divided into extrovert and introvert were analyzed.

The results show that animals as well as humans have a different individual response to stress. Studies conducted in the hotel show a tendency to adaptation i.e. median salivary cortisol level was lower each day, moreover, the

median cortisol level was lower in males than in females, which may suggest different stress resistance due to sex. Analysis of the relationship between motor lateralization and stress leads to the conclusion that dogs preferring the left paw were more susceptible to the stress stimulus because the serum cortisol levels of these individuals were significantly higher. Further research, not included in this PhD thesis, revealed that extroverted dogs are more susceptible to stress than introverted dogs.

Keywords: domestic dog, stress, motor lateralization, temperament

Wstęp

Pies w przeciągu kilkuset tysięcy lat znalazł wyjątkowe miejsce w Naszym życiu, to on towarzyszy człowiekowi najdłużej. Według najnowszych badań szacuje się, że udomowiony pies po raz pierwszy pojawił się w egzystencji człowieka ok. 33 000 lat temu (Ovodov et al. 2011). Stał się przede wszystkim towarzyszem, czworonożnym przyjacielem, ale także terapeutą, ratownikiem czy przewodnikiem. Na przestrzeni lat znacząco wzrosło znaczenie poziomu dobrostanu, a człowiek dąży do zapewnienia zwierzęciu komfortu nie tylko fizycznego, ale także psychicznego. Naukowcy starają się zgłębiać tajniki psiego umysłu, aby lepiej zrozumieć naturę psa.

Stres pełni bardzo ważną rolę w biologii zwierząt, pełni przede wszystkim funkcję adaptacyjną do wyzwań stawianych przez środowisko zewnętrzne (Chmelíková E. et al., 2020). W pracy z psami, bardzo ważną umiejętnością jest prawidłowe rozpoznawanie sygnałów wysyłanych przez zwierzę w różnych sytuacjach. Reakcja na stres często bywa odmienna osobniczo, dlatego tak ważna jest wnikliwa obserwacja danego zwierzęcia. Najważniejszymi wskaźnikami poziomu nasilenia stresu u ssaków są kortyzol i kortykosteron (Ulrich-Lai Y.M. and Herman J.P. 2009). Określanie nasilenia reakcji stresowej w odpowiedzi na różnorodne bodźce jest coraz bardziej popularne i stanowi pomocne narzędzie przy ocenie poziomu dobrostanu zwierząt, co może być wykorzystane do optymalnego zarządzania przestrzeniami dla zwierząt tj. miejsca szkolenia psów, hotele, salony pielęgnacyjne czy lecznice weterynaryjne (Williams i in., 2019).

Lateralizacja motoryczna, nazwana inaczej asymetrią mózgu określa preferencję łapy u danego psa. Jak pokazują badania psy analogicznie jak ludzie dzielą się na osobniki prawołapne i lewołpane. Do określenia stronności motorycznej wykorzystuje się wiele ustandaryzowanych testów m. in. test z taśmą umieszczoną na grzbiecie nosa psa (Quaranta i in. in., 2004), (Poyser i

in., 2006), (Batt i in., 2008), (Batt i in., 2009). Naukowcy zestawiają lateralizację motoryczną z wieloma niezależnymi czynnikami takimi jak płeć, wiek, sposób utrzymania, status immunologiczny, zjawisko adaptacji czy zaburzenia zachowania itp. Ponadto były liczne próby dowiedzenia, czy preferencja łapy ma wpływ na zdolności adaptacyjne psów, zdolności manualne, podatność na szkolenie, skłonności do odchyleń w prawidłowym zachowaniu (Wells i in., 2018), (Wells D.L. 2019).

W literaturze etologicznej znajdują się dwa pojęcia tj. temperament oraz osobowość, gdzie temperament stanowi wrodzoną część osobowości (John and Gosling, 2000). Jednoznaczne określnie temperamentu psa nastręcza wielu problemów, przede wszystkim dlatego, że pies nie komunikuje się z Nami werbalnie. Komunikacja opiera się na mowie ciała, która wymaga wnikliwej obserwacji oraz umiejętności jej interpretacji. W celu usprawnienia szacunkowej oceny temperamentu psa została stworzona ankieta skierowana do opiekunów zwierząt, z odpowiedzi której został utworzony algorytm oceny temperamentu psów (Karpiński M., et al., 2022).

Przedstawione zagadnienia formułują tematykę badawczą prac wskazanych jako niniejsza rozprawa doktorska.

Cel pracy:

Celem pracy jest ocena zależności pomiędzy poziomem stresu a lateralizacją motoryczną oraz ocena temperamentu u psów towarzyszących.

Cel główny był realizowany przez zadania cząstkowe:

- Ocena zjawiska adaptacji psów do stresujących warunków na podstawie oceny poziomu kortyzolu w ślinie psów przebywających co najmniej 3 dni w hotelu dla psów

- Ocena nasilenia reakcji na stres na podstawie poziomu kortyzolu w surowicy psów po zakończonych czynnościach lekarsko – weterynaryjnych

- Ocena lateralizacji motorycznej na podstawie ustandaryzowanego testu z taśmą przylepną

- Ocena zależności pomiędzy poziomem kortyzolu a lateralizacją motoryczną u badanych psów

- Opracowanie algorytmu oceny temperamentu psów

Materiał i metody

Badana grupa

Badania zostały przeprowadzone łącznie na 122 psach. Badania odbywały się w latach 2019 -2022 w dwóch częściach. Część pierwsza z udziałem 76 psów miała na celu zestawienie poziomu kortyzolu z lateralizacją motoryczną oraz zdolnościami adaptacyjnymi psów. Druga część przebiegła z udziałem 46 psów oraz ich opiekunów i polegała na opracowaniu algorytmu oceny temperamentu.

Wszystkie oznaczenia były wykonywane w trakcie trwania rutynowych czynności lekarsko – weterynaryjnych, za pisemną zgodą opiekunów zwierząt. Zwierzęta były kwalifikowane do badań pod względem zachowania oraz stanu zdrowia. Psy biorące udział w badaniach były zdrowe kliniczne, po pełnym badaniu klinicznym, z aktualną profilaktyką przeciwwirusową i przeciwpasożytniczą, nie wykazujące odchyleń od naturalnego garnituru zachowań. Badania przeprowadzono w hotelu dla zwierząt, w klinice i gabinecie weterynaryjnym oraz codziennym miejscu bytowania zwierząt.

Przebieg doświadczeń

Ocenę adaptacji na podstawie poziomu kortyzolu w ślinie wykonywano w hotelu dla psów w Lublinie. W badaniu wzięło udział 20 psów przebywających w hotelu dla zwierząt przez co najmniej trzy kolejne dni. W badanej grupie było 10 psów ras małych (5–10 kg) i 10 psów średniej wielkości (10,5–20 kg). W grupie było osiem suk i 12 psów w wieku od 2 do 8 lat. Badana grupa psów była zróżnicowana rasowo, jednak najpopularniejsze były psy rasy mieszanej. Psy biorące udział w badaniu były kwalifikowane

przez lekarza weterynarii oraz behawiorystę, przeszły pełne badanie kliniczne, posiadały aktualny kalendarz szczepień oraz profilaktykę przeciwpasożytniczą. Ponadto zwierzęta były zrównoważone pod względem zachowania, dobrze zsocjalizowane. Psy biorące udział w tym badaniu przebywały w hotelu w tym samym czasie i w tych samych warunkach. Wszystkie psy przebywały w indywidualnych boksach z dostępem do zewnętrznego wybiegu. To nie był pierwszy pobyt tych psów w hotelu dla zwierząt. Ślina była pobierana przez trzy kolejne dni, rozpoczynając od dnia przyjęcia, następnie po 24 i 48h po pierwszym pobraniu. Materiał pobierany był codziennie o stałej porze przez tą samą osobę z wykorzystaniem sterylnych wymazówek Salivette Cortisol firmy Sarstedt, Niemcy. Ślinę psów pobierano przez umieszczenie wacika w przedsionku jamy ustnej po wewnętrznej stronie policzków. W razie potrzeby stymulowano wydzielanie śliny za pomocą przysmaku (pies wąchał smakołyk, ale go nie dostawał).

Ocena lateralizacji motorycznej, temperamentu oraz pobieranie próbek krwi pełnej odbywało się w klinice weterynaryjnej dla zwierząt w Lublinie. Zwierzęta do tej części badań były kwalifikowane przez lekarza weterynarii na podstawie pełnego badania klinicznego oraz podstawowych badań morfologicznych i biochemicznych krwi. Grupa badawcza stanowiła łącznie 56 psów (34 samców i 32 samic) w przedziale 1-9 lat, w większości wielorasowe lub w typie rasy. Wszystkie zwierzęta były zdrowe klinicznie w trakcie trwania rutynowych czynności lekarsko – weterynaryjnych z aktualną profilaktyką. Materiał biologiczny (krew żylna) był pobierany przez lekarza weterynarii u psów po badaniu klinicznym (ok. 15-20 minut), któro stanowiło bodziec stresowy. Krew była pobierana do probówki o objętości 4ml, firmy PROFILAB do separacji surowicy. Lateralizacja motoryczna została określona u wszystkich badanych zwierząt. Wśród przebadanych zwierząt było 36 psów prawołpanych, 14 lewołpanych oraz 6 psów bez wyraźnej preferencji łapy.

Wszystkie metody zastosowane w badaniach były zgodne z Ustawą z dnia 15 stycznia 2015 r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. 2015 poz. 266) oraz ustawy z dnia 18 grudnia 2003 r. o zakładach leczniczych dla zwierząt (Dz. U. z 2019 r. poz. 24). Ponadto nie wymagały uzyskania zgody lokalnej komisji etyki, ze względu na nieinwazyjność badań (w przypadku śliny) oraz pozyskania (surowicy) w trakcie trwania czynności lekarsko – weterynaryjnych.

Ocena poziomu kortyzolu

Oceny poziomu kortyzolu dokonano w pobranym materiale biologicznym tj. ślinie oraz surowicy krwi.

Ślina: zebrany materiał odwirowano bezzwłocznie po pobraniu (3600 obr./min, 10 minut) i zamrożono w temperaturze -20°C. Po rozmrożeniu oznaczono stężenie kortyzolu. Do oznaczenia poziomu kortyzolu wykorzystano test immunoenzymatyczny do ilościowego pomiaru aktywnego wolnego kortyzolu w ślinie (test DRG Salivary Cortisol HS ELISA zgodnie z instrukcją producenta).

Surowica: próbkę pełnej krwi odwirowano w temperaturze pokojowej, 1500 obr./min przez 10 minut**.** Surowicę krwi po przeprowadzeniu podstawowych badań laboratoryjnych zamrożono w temperaturze -20°C do czasu oznaczeń. Badanie poziomu kortyzolu przeprowadzono metodą chemiluminescencji wzmocnionej enzymatycznie na analizatorze IMMULITE 2000 XPi SIEMENS oraz z użyciem DRG Salivary Cortisol HS ELISA zgodnie z instrukcją producenta.

Stężenie kortyzolu wyrażono w ng/ml.

Ocena lateralizacji motorycznej

Lateralizację motoryczną oceniano, obserwując, którą łapą pies oderwał kawałek taśmy samoprzylepnej z grzbietu nosa. Wykonano 50 powtórzeń zgodnie z ustandaryzowanym testem. Po zakończeniu testów taśmy, lateralizację obliczono ze wzoru $z = (R-0,5N)/\sqrt{(0,25N)}$, gdzie R oznacza liczbę użyć prawej łapy, a N oznacza sumę lewej i prawej łapy. Psy ze średnią z => 1,96 zostały sklasyfikowane jako z lateralizacją prawostronną, psy ze średnią z = <-1,96 zostały sklasyfikowane jako psy z lateralizacją lewostronną. Dla każdego z badanych zwierząt obliczono również parametr HI (wskaźnik ręczności), który dla psów z preferencją lewostronną wynosił 1,0, a dla psów z preferencją prawostronną -1,0 (Siniscalchi et al., 2008).

Ocena temperamentu

Ocenę temperamentu wykonano przy użyciu ankiety z wyszczególnionymi 24 cechami psów typowych dla ekstrawertyków i introwertyków. Cechy były zaznaczane na podstawie doświadczeń i obserwacji psa w neutralnych warunkach czyli np. podczas spacerów, zachowania w domu, relacji z innymi ludźmi i zwierzętami itd. Następnie ankiety były poddane wnikliwej analizie statystycznej.

Wzór ankiety:

tak	cecha	nie
	mądry	
	towarzyski	
	ciekawski	
	wesoły	

	dominujący	
	hałaśliwy	
	nieposłuszny	
	nadpobudliwy	
	sprytny	
	niecierpliwy	
	zuchwały	
	terytorialny	
	uparty	
	samotnik	
	agresywny	
	leniwy	
	łakomy	
	czujny	
	nieprzewidywalny	
	lękliwy	
	nieufny	
	niepewny	
	roztargniony	
	cichy	

Dla każdego psa zliczono z ankiety liczbę cech ekstrawertycznych LE (należących do skupienia 1) oraz liczbę cech introwertycznych LI (należących

do skupienia 2). W celu zbudowania modelu oceny temperamentu przeprowadzono regresję logistyczną ze zmiennymi niezależnymi LE i LI oraz zmienną zależną dychotomiczną (1-ekstarwertyk, 0-introwertyk).
Otrzymano model regresji logistycznej:

$$(Y = 1|LE = x_1, LI = x_2) = \frac{e^{a_0 + a_1 x_1 + a_2 x_2}}{1 + e^{a_0 + a_1 x_1 + a_2 x_2}}$$

Na podstawie zbudowanego modelu regresji logistycznej podstawiając do prawej strony równania dla każdego badanego psa liczbę cech ekstrawertycznych LE = x_1 $LE = x_1$ oraz liczbę cech introwertycznych LI = x_2 $LI = x_2$ oraz parametry a_0, a_1, a_2 z modelu, otrzymamy wartość prawdopodobieństwa, że pies ma osobowość ekstrawertyczną. Jeżeli otrzymane prawdopodobieństwo P>0,5, to klasyfikujemy go do grupy ekstrawertyków, jeśli zaś P<0,5, to klasyfikujemy go do grupy introwertyków. Model pozwala więc na podstawie ankiety klasyfikować psy do odpowiedniej grupy temperamentu.

Analizy statystyczne

Analizę statystyczną dla określenia zjawiska adaptacji u psów wykonano przy użyciu pakietu Statistica 13.1 z wykorzystaniem testu Shapiro – Wilka, testu U-Rang Manna – Whitneya oraz analizy wariancji ANOVA Kruskala – Wallisa. Do opisu rozkładu wykorzystano wartości średniej tj. mediany i kwartyli.

Analizę statystyczną dla oceny korelacji pomiędzy poziomem kortyzolu, a lateralizacją motoryczną wykonano przy użyciu Pakietu Statistica 13.1. Zgodność rozkładu badanych cech z rozkładem normalnym oceniano testem Shapiro-Wilka. W celu porównania średnich wartości zmiennych zależnych przeprowadzono nieparametryczną analizę wariancji Kruskala-Wallisa

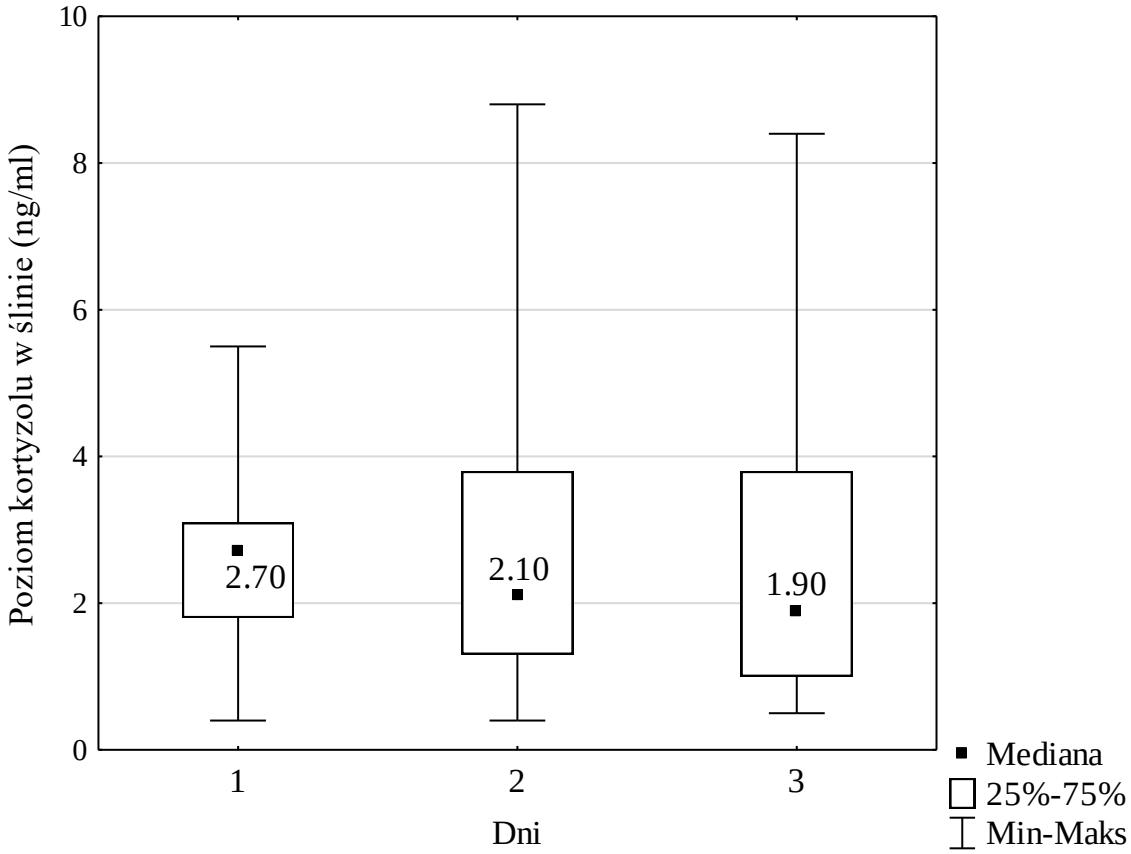
ANOVA. Do określenia wpływu płci na preferencje łap zastosowano test $\chi 2$. Istotność różnic między zmiennymi zależnymi określono za pomocą oznaczeń literowych.

Algorytm oceny temperamentu u psów został opracowany za pomocą metody aglomeracji, przy wykorzystaniu odległości 1-r Pearsona i metody wiązania Warda.

Omówienie wyników

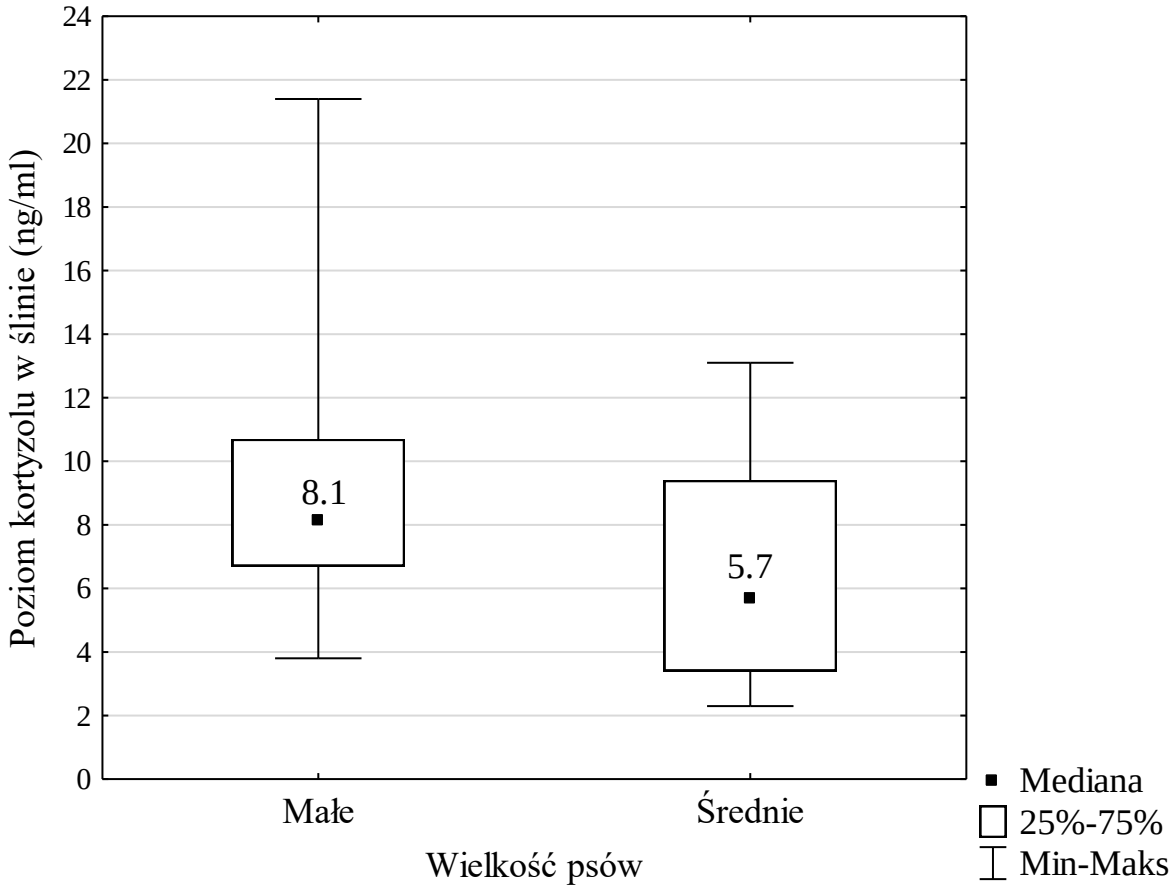
Przeprowadzone badania miały zweryfikować czy zwierzęta maja zdolność adaptacji do nowych warunków otoczenia oraz czy uwarunkowania genetyczne takie jak lateralizacja motoryczna mają wpływ na różnice w poziomie kortyzolu u psów wystawionych na czynniki stresujące (czynności lekarsko – weterynaryjne). Ponadto opracowano algorytm oceny temperamentu, aby w przyszłości w dostępny i przystępny sposób określać temperament psów. W dalszej nieopublikowanej części badań starano się, także wykazać korelację pomiędzy poziomem kortyzolu, a temperamentem jako wrodzoną częścią osobowości.

Uzyskane wyniki badań wskazują, że zwierzęta posiadają skłonność do przystosowania się do stresujących warunków jakimi był pobyt w hotelu dla zwierząt, który wiązał się ze zmianą rytmu dnia, nowymi odgłosami, kontaktem z innymi zwierzętami oraz rotacją personelu. Mediana poziomu kortyzolu w ślinie u psów wynosiła 2,7 ng/ml przy przyjęciu do hotelu, 2,1 ng/ml po 24 godzinach i 1,9 ng/ml 48 godzin po przyjęciu (Rycina 1).



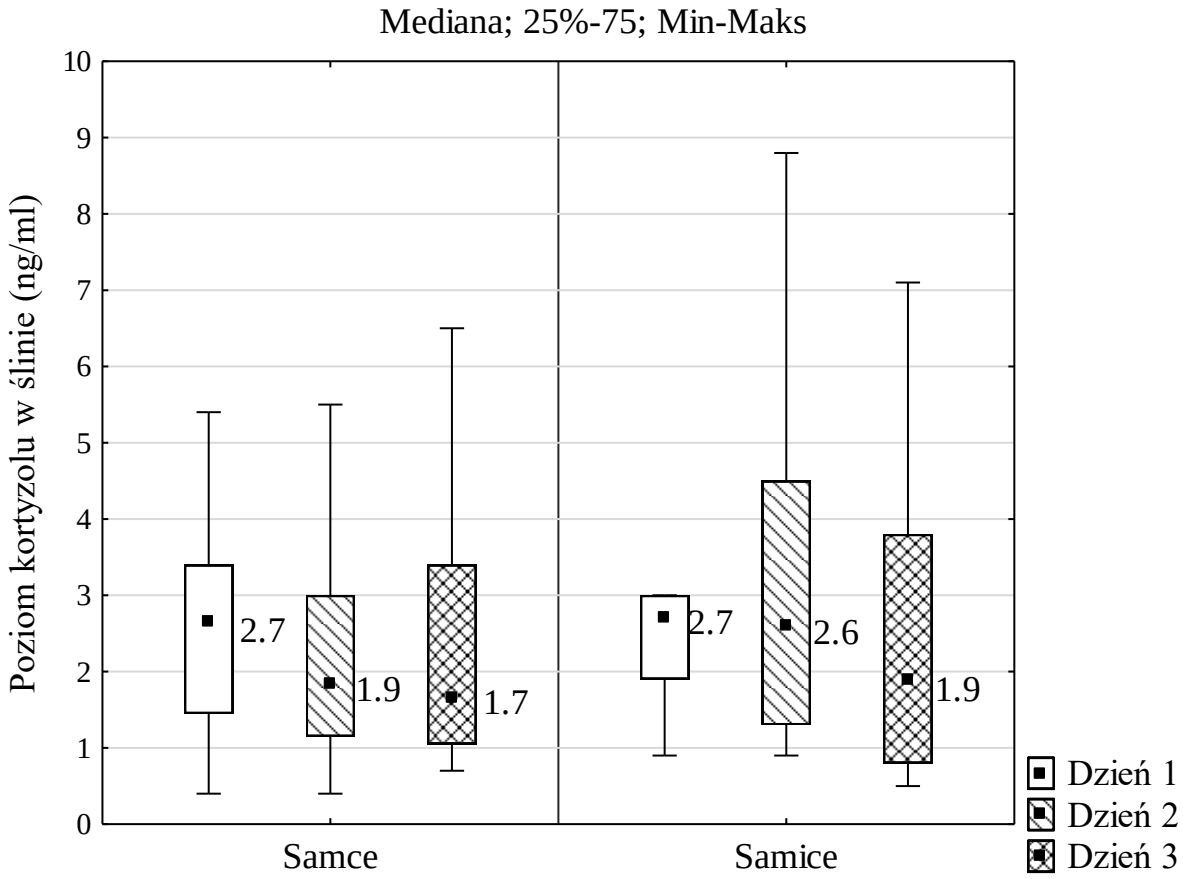
Rycina 1. Rozkład poziomu kortyzolu w ślinie w kolejnych dniach pobytu psów w hotelu (Kruskal-Wallis ANOVA: $\chi 2 = 1{,}689$; df= 2; $p = 0{,}430$).

Porównując poziom kortyzolu u psów z podziałem na wielkość, można zauważyć pewną tendencję do większego stopnia adaptacji u psów większych tj. mediana poziomu kortyzolu w ślinie w kolejnych dniach wynosiła 2,2 ng/ml, 1,7 ng/ml i 1,2 ng /ml, natomiast u psów małych ras 2,8 ng/ml, 2,9 ng/ml i 2,4 ng/ml (Rycina 2).



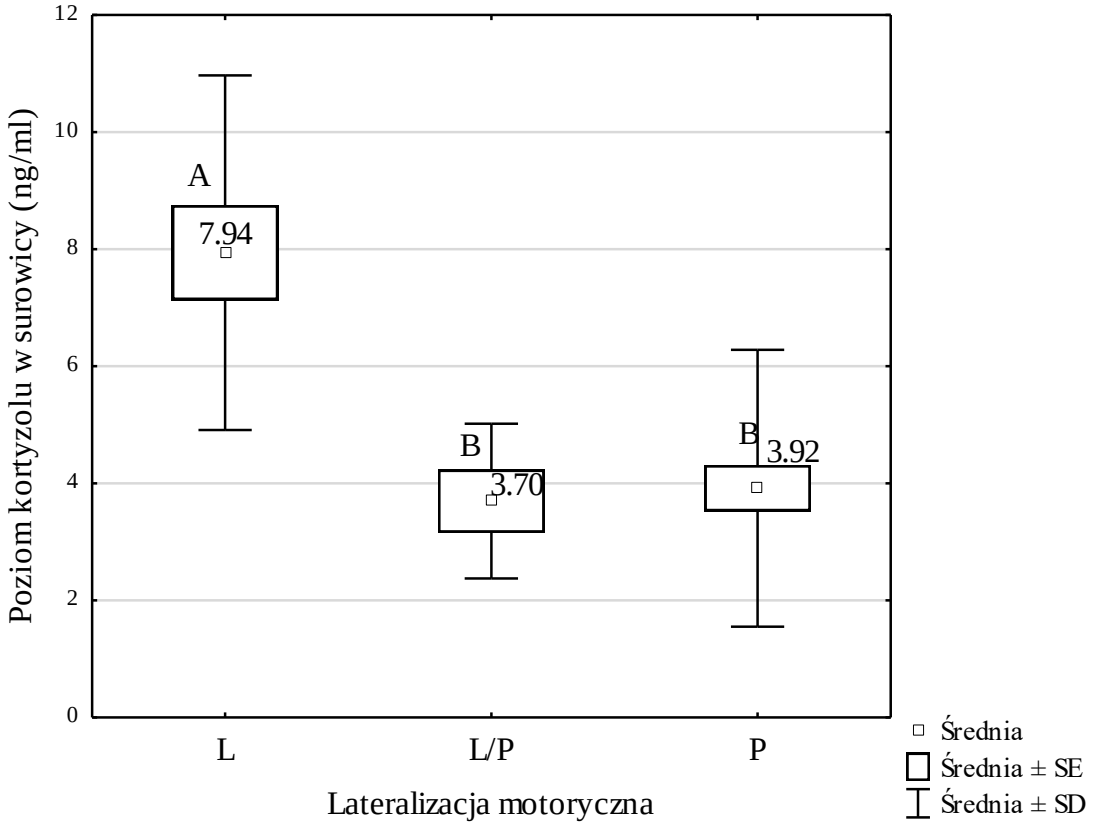
Rycina 2. Rozkład poziomu kortyzolu w kolejnych dniach z uwzględnieniem wielkości badanych psów (Kruskal-Wallis ANOVA: Small – $\chi 2 = 1.187$; df= 2; $p = 0.552$; Medium – $\chi 2 = 0.800$; df= 2; $p = 0.670$).

W zależności od płci psów mediana poziomu kortyzolu u samców wynosiła: pierwsza doba – 2,7 ng/ml, druga doba – 1,9 ng/ml, trzecia doba – 1,7 ng/ml, a u suk: pierwsza doba 2,7 ng/ml, drugi dzień – 2,6 ng/ml, trzeci dzień – 1,9 ng/ml (Rycina 3). Obserwowane różnice nie były istotne statystycznie, niemniej jednak można zauważyć pewną tendencje do wyższego poziomu kortyzolu u suk.



Rycina 3. Rozkład poziomu kortyzolu w ślinie w kolejnych dniach pobytu w hotelu w zależności od płci (Kruskal-Wallis ANOVA: mężczyźni – chi2 = 2000; df= 2; p = 0,368; suki – chi2 = 0,382; df= 2; p= 0,826).

Badania pozwoliły na stwierdzenie, że cechy wrodzone mają wpływ na odmienne nasilenie reakcji stresowej. Zestawiając lateralizację motoryczną z poziomem kortyzolu, można stwierdzić, że średni poziom kortyzolu we krwi u psów z preferencją lewej łapy był istotnie wyższy i w badanej grupie 56 wynosił i wynosił 7.94 ug/dl. U psów z prawostronną średni poziom kortyzolu wyniósł 3.92 ug/dl a u psów bez wyraźnej preferencji łapy 3.7 ug/dl (Rycina 4).



Rycina 4. Rozkład poziomu kortyzolu w surowicy u psów w zależności od lateralizacji motorycznej (ANOVA Kruskala-Wallisa, $\chi 2=13,730$; $p=0,001$)

W tej samej grupie psów zbadano również poziom tyroksyny fT4 i odnotowano jej wyższy poziom u psów z lewostronną preferencją łapy, podobnie jak poziom kortyzolu, co może wskazywać na ich wzajemną korelację. W psów lewołapnych jej średnie stężenie wynosiło 1,95 ng/dl, w psów z prawostronną lateralizacją 1,56 ng/dl i obustronną 1,01 ng/dl. Niemniej jednak uzyskane wyniki mogą jedynie sugerować wzajemną korelację, ze względu na brak statystycznie istotnego wyniku.

Nie odnotowano istotnie statystycznych różnic w poziomie kortyzolu, a lateralizacją pomiędzy płcią męską i żeńską, a także u psów poddanych i nie poddanych zabiegowi kastracji.

Podsumowanie i wnioski

Na postawie uzyskanych wyników, można stwierdzić, że:

- Psy posiadają zdolności adaptacyjne do stresujących warunków w trakcie przebywania w hotelu dla psów
- Płeć psów nie ma istotnego wpływu na poziom reakcji stresowej, kształtowanie temperamentu oraz asymetrii mózgu
- Wrodzone skłonności psów m.in. stronność odgrywają znaczącą role w sposobie radzenia sobie ze stresem, czego dowodem jest wyższy poziom kortyzolu u psów lewołapnych
- Otrzymany algorytm oceny temperamentu może być wykorzystywany w testach predyspozycji psów.

Aby lepiej zrozumiem tajniki psiego umysłu warto znać jego preferencję łapy oraz rodzaj temperamentu i skutecznie zapobiegać sytuacjom stresowym lub spróbować łagodzić poziom stresu, aby zachować poziom dobrostanu na odpowiednim poziomie. Otrzymane wyniki mogą posłużyć do szybkiej oceny podatności psów na stres (w oparciu o preferowaną kończynę i temperament) i w efekcie zindywidualizowanych działań w kontekście jego łagodzenia.

W niepublikowanej dalszej części badań, wyniki ujawniły dodatnią korelację pomiędzy poziomem kortyzolu w surowicy psów, a temperamentem. Psy z temperamentem ekstrawertycznym cechowały się statystycznie wyższym poziomem kortyzolu, niż psy introwertyczne. Co przemawia, za koniecznością

przegrupowywania zwierząt w takich miejscach jak hotele czy szkoły dla psów, aby zadbać o najwyższy poziom dobrostanu.

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Dogs' Stay in a Pet Hotel – Salivary Cortisol Level and Adaptation to New Conditions

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ABSTRACT

The aim of this study was to assess salivary cortisol level in dogs during admission and a three-day stay in a pet hotel. The study involved 20 dogs admitted to a pet hotel for a stay lasting for at least three consecutive days. The study group consisted of 10 small dogs and 10 medium-sized dogs, eight females and 12 males. Saliva was collected each day to assess cortisol levels. A stay in a hotel is a stressful situation for dogs, as evidenced by the increase in the salivary cortisol level. The highest salivary cortisol level was observed on the day of admission to the hotel. The median salivary cortisol level in the dogs on the consecutive days was 2.7 ng/ml on admission, 2.1 ng/ml after 24 hours, and 1.9 ng/ml after 48 hours. Depending on the size of the dog, the median salivary cortisol level was 2.8 ng/ml, 2.9 ng/ml and 2.4 ng/ml in the small dogs and and 2.2 ng/ml, 1.7 ng/ml and 1.2 ng/ml in the medium sized dogs on the consecutive days.

KEYWORDS

Dog; pet hotel; salivary cortisol level; adaptation; behavior

Introduction

The two biological systems involved in the organism stress response are the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. An effect of activation of the former is increased secretion of catecholamines, i.e. adrenaline and noradrenaline. In turn, the synthesis and secretion of glucocorticoids into the blood is an effect of activation the HPA axis. Glucocorticoids are steroid hormones secreted by the adrenal cortex. Steroid hormones, including cortisol, are measured in dogs for diagnostic purposes of endocrine or dermatological diseases, but also to control the severity of the stress (Wenger-Riggenbach et al., 2010). The most common diseases in which cortisol levels increase are hyperadrenocorticism (Cushing's syndrome), cancer of the adrenal gland, thyroid and pituitary gland. Increased cortisol levels may also be associated with obesity, high protein diet or high physical activity (Hennessy et al., 2002). In the group of tested dogs, the stress reaction and cortisol ejection could have occured as a result of separation from owner, new environment, the presence of new people and other animals. What's more a stress factor in a hotel can be noise, changing the rhythm of the day, new smells or locked in a cage.

In dogs, likewise in humans, cortisol, which is referred to as a stress hormone, is secreted in the largest amount. The biological material used to determine its level is usually blood. However, biological materials collected in a noninvasive way, such as saliva (Dreschel & Granger, 2009), urine (Stephen & Ledger, 2006), feces, hair (Accorsi et al., 2008), and even nails (Nejad et al., 2016) are increasingly being used. Currently, saliva is the most convenient research material for assessing the level of steroid hormones (Dziurkowska & Wesołowski, 2010). The salivary cortisol concentration is an extremely useful stress biomarker (Hellhammer, Wust, & Kudielka, 2009).

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The aim of the study was to determine the level of stress based on the salivary cortisol concentration in dogs staying in a hotel for animals (kennels) and to assess their adaptation to hotel conditions.

Material and methods

The study involved 20 dogs staying in an animal hotel for at least three consecutive days. In the study group, there were 10 dogs representing small breeds (5–10 kilograms b.w.)and 10 medium-sized dogs (10,5–20 kilograms b.w.). There were eight females and 12 males with an average age of five years (between 2 and 8 years ago). The group of dogs that was tested varied in terms of breed, however, the most popular weremix breed dogs.

Dogs were selected for the study based on their emotional state. They were well socialized dogs that did not display any signs of aggression or anxiety toward other dogs or strangers. All dogs were kept in home conditions (block of flats) on a daily basis. At the hotel reception all dogs were clinically healthy and their vaccinations against rabies and infectious diseases as well as antiparasitic prophylaxis were up to date. The dogs participating in the study stayed at the hotel at the same time and under the same conditions with regards to walking, feeding, crates cleaning as well as other environmental conditions such as temperature, humidity, noise. All dogs stayed in individual crates with access to open-air catwalk. The staff rotation was constant throughout the entire study period. This was not dogs' first stay in the hotel.

The biological material for the study was saliva, in which cortisol levels were determined. It was collected using Salivette Cortisol synthetic swab tubes from Sarstedt, Germany. The saliva of the dogs was collected by rubbing a cotton pad on the lips and the inside of the cheeks. If necessary, saliva secretion was stimulated with the help of a treat. No circadian cortisol secretion rhythm was detected in the dogs (Johnston & Mather, 1978; Koyama, Omata, & Saito, 2003; Thun, Eggenberger, & Zerobin, 1990; Wenger-Riggenbach et al., 2010); hence, the first saliva sample was taken when the dog was admitted to the hotel, regardless of the time. However, subsequent samples were taken 24 hours and 48 hours after the first collection. The collected material was centrifuged immediately after collection (3600 rpm, 10 minutes) and frozen at −20°C. After thawing, the cortisol concentration was determined. For research was used an enzyme immunoassay for the quantitative measurement of active free cortisol in saliva (DRG Salivary Cortisol HS ELISA assay). The lowest detectable level of Cortisol that can be distinguished from the Zero Standard is 0.537 ng/ml. The range of the assay is between 0.537– 80 ng/ml. The procedures were carried out in accordance with the manufacturer's instructions. The cortisol concentration was expressed in ng/ml.

Statistical analysis of the results was carried out using the Statistica 13.1 statistical package. The normality of the distribution of the analyzed traits was assessed with the use of the Shapiro-Wilk test. Since the distribution of the traits was significantly different from normality, nonparametric (rank) tests were used to assess the significance of differences between the distributions. The Mann-Whitney U rank test was used to compare the two groups. In turn, to compare a larger number of groups, a nonparametric analysis of variance i.e. Kruskal-Wallis ANOVA and pairwise multiple comparisons of the mean ranks were applied. A measure of position of the mean value, i.e. the median and quartiles, were used to describe the distributions. For selected parameters, the distribution of the analyzed traits in individual days is illustrated on categorized frame graphs based on the measures of position (median, quartiles).

Results

The median salivary cortisol level in the dogs was 2.7 ng/ml on admission to the hotel, 2.1 ng/ml after 24 hours, and 1.9 ng/ml 48 hours after admission (Figure 1). Depending on the size of the dog, the median salivary cortisol level on the consecutive days was 2.8 ng/ml, 2.9 ng/ml and 2.4 ng/ml in the small dogs and and 2.2 ng/ml, 1.7 ng/ml and 1.2 ng/ml in the medium sized dogs (Figure 2). Depending

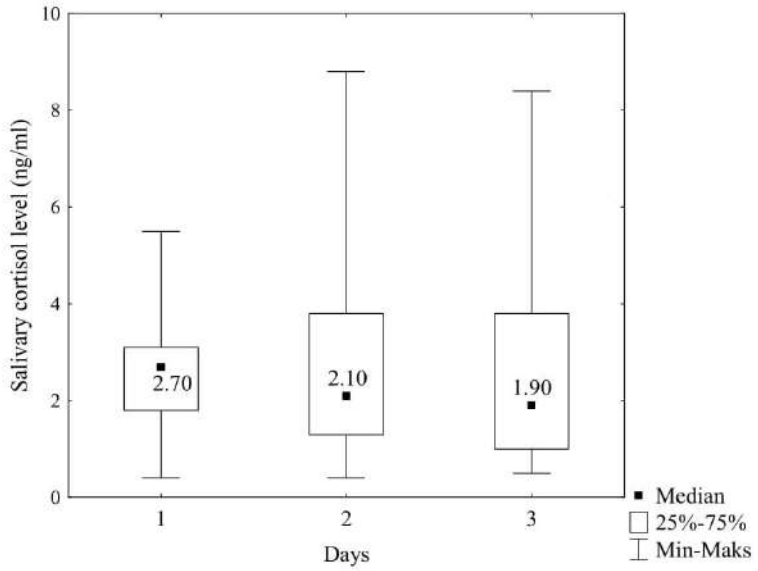

Figure 1. Salivary cortisol level in the following days of stay at the hotel (Kruskal-Wallis ANOVA: $\chi^2 = 1.689$; $df = 2$; $p = 0.430$).

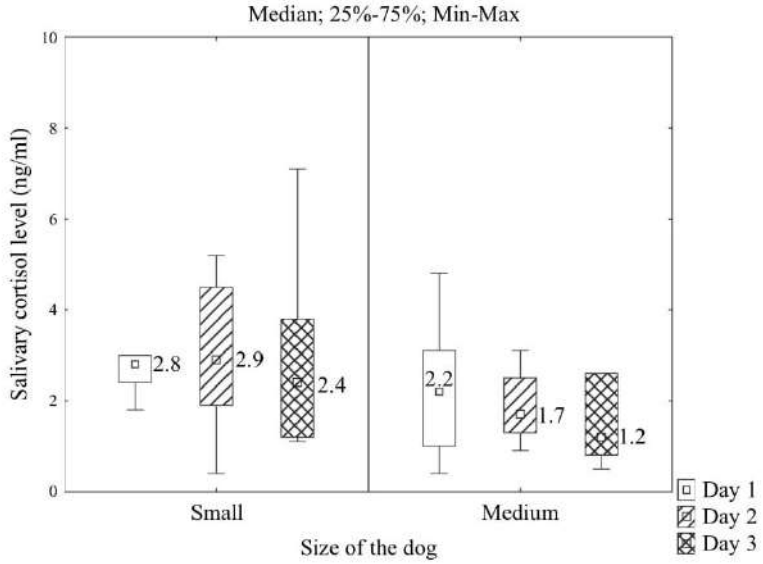

Figure 2. Salivary cortisol level in the following days of stay at the hotel depending on the dog's size (Kruskal-Wallis ANOVA: Small – $\chi^2 = 1.187$; $df = 2$; $p = 0.552$; Medium – $\chi^2 = 0.800$; $df = 2$; $p = 0.670$).

on the sex of the dogs, the median cortisol level in males was: first day – 2.7 ng/ml, second day – 1.9 ng/ml, third day – 1.7 ng/ml and in females: first day 2.7 ng/ml, second day – 2.6 ng/ml, third day – 1.9 ng/ml (Figure 3). The observed differences were not statistically significant.

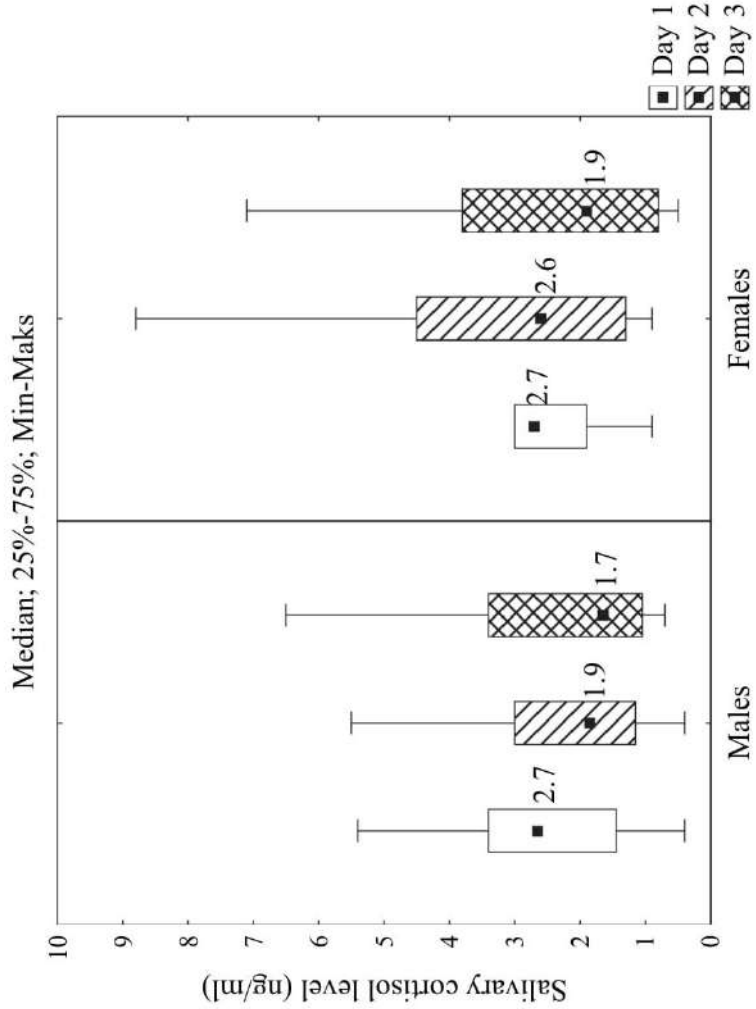

Figure 3. Salivary cortisol level in the following days of stay at the hotel depending on the dog's sex (Kruskal-Wallis ANOVA: Males – χ^2 = 2.000; df= 2; p = 0.368; Females – χ^2 = 0.382; df= 2; p= 0.826).

Discussion

The median cortisol level in the saliva of dogs on each subsequent day in the hotel was higher than the mean cortisol level in the saliva of dogs tested in neutral conditions by other authors. A level of 0.48 ± 0.3 ng/ml was reported by Wenger-Riggenbach et al. (2010), 1.89 ng/ml by Beerda, Schilder, Van Hooff, De Vries, and Mol (1998), and 1.7 ng/ml by Vincent and Michell (1992). A stay in a hotel is therefore a stressful situation for dogs, as evidenced by the increase in the salivary cortisol levels observed in the present study. However, it seems that the stress associated with staying in a hotel is lower than in dogs used in dog therapy, in which the mean level of cortisol measured before the therapeutic session ranges from 3–4 ng/ml (Glenk et al., 2014). The examined dogs were also definitely less stressed than dogs admitted to the veterinary clinic for planned procedures, in which Hekman, Karas, and Dreschel (2012) found a mean cortisol level of 8.7 ng/ml. The relatively low level of stress in the dogs examined in the present study may be related to the fact that it was not their first stay in this place.

Adaptation to stressors is an important aspect in behavioral research. In conducted researches the moment of admission to the hotel and separation from the owners was the most stressful for dogs. Each subsequent day, the mean cortisol level was lower than that on the previous day (Figure 1). The obtained result, however, is not statistically significant, so it cannot be clearly determined whether the dogs participating in the research have adapted to the new conditions.

The level of cortisol in the examined dogs in the following days of stay in the hotel did not differ significantly in males and females. Differences between cortisol levels in dogs depending on the sex were not reported by Reimers, Lawler, Sutaria, Correa, and Erb (1990), Koyama et al. (2003), and Sandri, Colussi, Perrotta, and Stefanon (2015).

Higher cortisol levels were observed in the small than medium-sized dogs (Figure 2). The results obtained are not statistically significant, but they are consistent with the results presented by other authors. Higher cortisol levels in small dogs compared to medium, large, and giant dogs were detected by Reimers et al. (1990) and Sandri et al. (2015). The authors try to interpret the obtained results carefully. We are aware that the concentration of cortisol in saliva can be a reflection of many factors affecting animal welfare. Moreover, inter-individual differences in the level of the cortisol related to age, stress tolerance, diet, exercise or co-morbidities are often cited by the authors of Carrier, Cyr, Anderson, and Walsh (2013), Cobb, Iskandarani, Chinchilli, and Dreschel (2016) as restrictions on the use of cortisol levels to determine the intensity of a stress.

Conclusions

A stay in a hotel is a stress factor for dogs, but their median salivary cortisol level is only slightly higher than the cortisol level in relaxed dogs reported by other authors. Based on the results obtained, it cannot be determined whether the dogs have adapted to the hotel conditions. There were no significant differences in cortisol levels in males and females. It is possible that the lack of statistically significant differences in the results obtained in the present study is associated with the short period of the studyand the lack of segregation of the tested animals in terms of personality, previous experience, diet or lifestyle. The concentration of cortisol in saliva can be a reflection of many factors affecting animal welfare. Therefore, it seems necessary to conduct further studies on a larger group and over a longer time to confirm the observed relationships.

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The Relationship Between the Motor Lateralization and the Concentration of Cortisol and Tyrosine in Dogs Exposed to Stress During Veterinary Activities

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ABSTRACT

The aim of the study was to assess the relationship between the motor lateralization in dogs and the concentration of cortisol and tyrosine in their plasma during a visit to a veterinary clinic. The research group consisted of 56 dogs. Motor lateralization was tested by of an adhesive tape test. The stress intensity was assessed basing on the levels of cortisol and ft4 in the serum blood. The statistical analysis revealed that in the group of 56 dogs there were 14 left-pawed dogs, 36 right-pawed dogs, and 6 dogs did not show particular paw preference. The average cortisol levels in particular groups were as follows: 7.94 ug/dl, 3.92 ug/dl and 3.7 ug/dl, whereas the level of tyrosine in the subjects that demonstrated left-sided lateralization (mean ± SE) (1.95 ± 0.46 ng/dl) for those with right-side lateralization (1.56 ± 0.23 ng/dl) and ambilateral dogs (1.01 ± 0.22 ng/dl). The statistical calculation of Pearson's χ^2 showed a significant relationship between the sex and the lateralization ($\chi^2 = 6.238$, df = 2, p = 0.0442).

KEYWORDS

Dog; motor lateralization; stress; cortisol; tyrosine

Introduction

The lateralization (that is: the asymmetric specialization of the brain hemisphere) is a phenomenon often described and studied in both humans and various animal species in the processing of information that reaches the brain. It is believed that the asymmetry develops as the body grows, acquires the ability to control and coordinate movements, and as the level of perception increases (Corballis, 2009). Research that was conducted so far leaves no doubt that brain lateralization also affects animal behavior, because research conducted on various animal species indicated that the left hemisphere of the brain is more involved in the control of invariant stimuli or repetitive actions (MacNeilage, Rogers, & Vallortigara, 2009), while the right hemisphere specializes in quality of emotional responses such as aggression or fear (Marshall-Pescini, Barnard, Branson, & Valsecchi, 2013) and reacting to new stimuli and behavior related to escape (Siniscalchi, McFarlane, Kauter, Quaranta, & Rogers, 2013). In the case of dogs, establishing relationships between functional asymmetry and adaptation strategy (Wells, Hepper, Milligan, & Barnard, 2018). behavioral reactivity is crucial in terms of the animals' well-being. Lateralization in dogs is most often researched by determining the dominance of one of the forepaws in the "Kong ball test" (Wells, 2003), (Marshall-Pescini et al., 2013). or the adhesive tape test-motor lateralization (Quaranta, Siniscalchi, Frate, & Vallortigara, 2004), (Poyser, Caldwell, & Cobb, 2006), (Batt, Batt, Baguley, & McGreevy, 2008), (Batt, Batt, Baguley, & McGreevy, 2009).

The issue of the connection between motor lateralization and dogs' emotional well-being has received much attention in the previous research. The effect of paws bias on animal temperament and individual responses to new stimuli (Siniscalchi, Sasso, Pepe, Vallortigara, & Quaranta, 2010), as

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well as the relationship between paws bias and sex (Wells et al., 2018), (McGreevy, Brueckner, Thomson, & Branson, 2010). Studies conducted by Siniscalchi, d'Ingeo, Fornelli, and Quaranta (2016) and Quaranta et al. (2004) have demonstrated a relationship between paw preference and sex at the population level, as males exhibited a left paw preference and females preferred using the right paw. Similar results were presented by Wells et al. (2018) and McGreevy et al. (2010). It should be noticed that many studies on dog's behavior did not find this sex difference in lateral behavior (Branson, 2006), (Schneider, Delfabbro, & Burns, 2013).

Stress involves many reactions in the body that allow the animal to adapt to a new situation (Moberg & Mench, 2000). The nervous and endocrine systems are activated by the stress stimuli and that in consequence changes the animal's behavior. What is very characteristic in the stress response is the release of glucocorticoids from the adrenal cortex as a result of the activation of the HPA axis (Hennessy, Davis, Williams, Mellott, & Douglas, 1997). When assessing the levels of stress during various research one of the most interesting and helpful glucocorticoids that is taken into consideration is cortisol. Its increased concentration is associated with the feeling of anxiety in many species, so it can be perceived as a good indicator of the animals' response to a new, stressful situation (Haverbeke, Diederich, Depiereux, & Giffroy, 2008). The assessment of cortisol levels is an effective method for testing both acute and chronic agitation and stress in dogs (Bennett & Hayssen, 2010). In addition to glucocorticoids, thyroid hormones are also involved in stress regulation. Thyroid hormones are responsible for stimulating metabolism and thermogenesis in the muscles (Reimers, Lawler, Sutaria, Correa, & Erb, 1990).

The study of emotional reactivity in response to various stimuli is increasingly popular in behavioral medicine and is helpful when assessing animal welfare. Moreover, it provides an opportunity to implement optimal changes in space management in veterinary clinics (Williams, Carroll, & Montrose, 2019). The body is constantly in the cycle of stimulation and suppression (allostatis). Not every stimulation causes stress, but every stress is a stimulation (Haubenhofer & Kirchengast, 2006). It is difficult to assume that stress negatively impacts the animal's body at all times and significantly reduces the level of well-being. However, the effective management of stress in veterinary clinics is worth implementingfor example, because it creates a possibility of avoiding dangerous situations caused by scared dogs that undergo various medical procedures.

The present study aimed to assess the relationship between canine motor lateralization and the intensity of the stress response. Plasma cortisol level was considered an indicator of acute stress during a visit to the veterinary clinic. The authors sought to determine whether animals with right or left paw preference would be more susceptible to stress. It was determined what percentage in the group of tested animals showed a preference for the left or right paw, and what was the percentage of right and left-footed animals in the male and female groups. Moreover, it was investigated whether there is a stress-gender correlation, i.e., whether the stress level will be higher in males or females. Thyroxine was determined as an additional parameter in order to correlate its level with the level of cortisol and confirm or exclude the relationship of thyroxine with stress reaction.

Materials and methods

Animals

The research group consisted of 56 dogs (24 males and 32 females) ranging from 0.5 to 9 year old. The dogs were of various sizes. All study subjects belonged to the clients of a veterinary clinic. All animals were healthy and have been receiving regular antiparasitic prophylaxis. The dog owners were interviewed to check if the dogs have shown any behavioral disorders such as excessive aggression or fearfulness but it was not stated in any of the interviews. As part of the veterinary intervention, the dogs were to be castrated, or they were to receive dental treatment. Animals were taken for surgery in the morning, after proper dietary preparation consisting in a 12-hour fast and a 24-hour break from intense physical exertion.

Test procedure

Before qualifying for research animals underwent a full, routine clinical examination conducted by a veterinarian, and blood laboratory tests were performed which included a complete morphological and biochemical analysis. Blood was collected as part of veterinary medical activities, as there was no interference with animal well-being and the researchers obtained the informed consent by the owners hence permission from Commission for Experiments on Animals in Poland is not requied for this research. Cortisol and fT4 levels were determined from serum in parallel with blood tests that were routinely performed before a medical procedure that involved anesthesia and surgery. The level of cortisol was determined once from a blood sample collected before the treatment. The values of the cortisol level of individual animals were later compared to referenced values. After qualifying for our research obtaining consent from the owners, the owners were shown twice how to perform the tape test on the bridge of the dog's nose and how to correctly interpret the animal's behavior. The owners declared the remaining 48 repetitions of the test would be performed at home within a month.

Paw preference assessment

The motor lateralization was assessed by observing which paw the dog used to remove the piece of the adhesive tape from the bridge of its nose. After completion of the tape test results the lateralization was calculated using the formula $z = (R - 0.5\ N)/\sqrt{(0.25\ N)}$, where R signifies the number of R paw uses and N signifies the sum of L plus R paw uses. Dogs with mean $z = > 1.96$ were classified as right-pawed, dogs with mean $z = <-1.96$ were classified as left-pawed, whereas dogs with intermediate z values were classified as ambilateral. For each of the tested animals, the parameter HI (handedness index) was also calculated, which for dogs with a left-sided preference was 1.0, and for dogs with a right-sided preference −1.0. A HI score of 0 indicates equal use of the L and R paws (Siniscalchi, Quaranta, Rogers, & Lauwereyns, 2008).

Cortisol and fT4 concentration management

In order to confirm or exclude the relationship between the increase in reactivity/stimulation and cortisol concentration and lateralization, fT4 concentration was examined, which is in a negative feedback with cortisol (Rijnberk & Kooistra 2010). Examination of cortisol and fT4 levels was performed using an enzymatically enhanced chemiluminescence method on the IMMULITE 2000 XPi SIEMENS analyzer. To obtain blood serum, the whole blood sample was centrifuged at room temperature, 1500 rpm for 10 minutes.

Statistical analysis

The statistical analysis of the obtained results was carried out using the Statistica 13.1 statistical package. The compatibility of the distribution of the examined features with the normal distribution was assessed by the Shapiro–Wilk test. Because the distributions of the analyzed dependent variables (cortisol concentration, thyroxine concentration) did not have a normal distribution, non-parametric (rank) tests were used to test the significance of differences between their distributions. A non-parametric analysis of variance – Kruskal–Wallis ANOVA was carried out to compare the mean values of the dependent variables. The result of the multiple comparison test is displayed on box charts showing arithmetic means, SE and SD. To compare the differences between the percentages of individuals with specific motor lateralization in all dogs, a test for two structure indicators was used. The χ^2 compliance test was used to determine the effect of gender on paw preference. The significance of differences between dependent variables was determined using the so-called letter designations. Groups with a common letter in the letter designation do not differ significantly (they

belong to the same homogeneous group), while there are significant differences between groups that do not have the same letter in the designation. Spearman's correlation coefficient was used to determine the relationship between cortisol concentration and thyroxine concentration (fT4).

Results

Calculations of the preference index (z) showed that most of the dogs tested had a clear preference for using the right paw 64% ($z > +1,96$), the percentage of dogs that displayed left-paws was 25% ($z < -1,96$), the fewest individuals were ambilateral 11% ($-1,96 < z < +1,96$), (Table 1).

The mean absolute value of preferences (HI) in the right and ambiprefent individuals was high and comparable, while in the ambilateral individuals it was characterized by a very low value.

In the group of 56 dogs, 14 subjects presented left paw preference (25%), 36 right paw preference (64%), and 6 were found to be ambilateral (11%) during the test. The number of individuals with right-sided preference was significantly higher compared with the number of individuals with left-sided preference and ambilateral individuals (Figure 1).

In the group of 32 female dogs, the vast majority (78%) showed right-sided motor lateralization (25 subjects), 16% left-sided (5 subjects), and the remaining 6% were ambilateral (2 subjects). Among male dogs, almost half (46%) showed right-sided lateralization (11 subjects), 38% left-sided (9 subjects) and 17% ambilateral paw preference (4 subjects). Pearson's χ^2 statistical calculations showed a significant relationship between gender and lateralization ($\chi^2 = 6.238$, df = 2, p = 0.0442) that is, in the group of females, the vast majority showed a right-paw preference compared to the male group (Figure 2).

Table 1. The range is the value of the preference index (z) (tape test) and the absolute value of the preference force (HI) for both paws.

Lateralization	z		HI (the absolute value)	
	average	range	average	range
L (n = 14)	−3,61	−4,5; −2,3	0,51	0,4; 0,6
P (n = 36)	3,55	2,5; 3,6	0,50	0,3; 0,7
L/P (n = 6)	−0,24	−0,9; 0,0	0,02	0,02–0,1

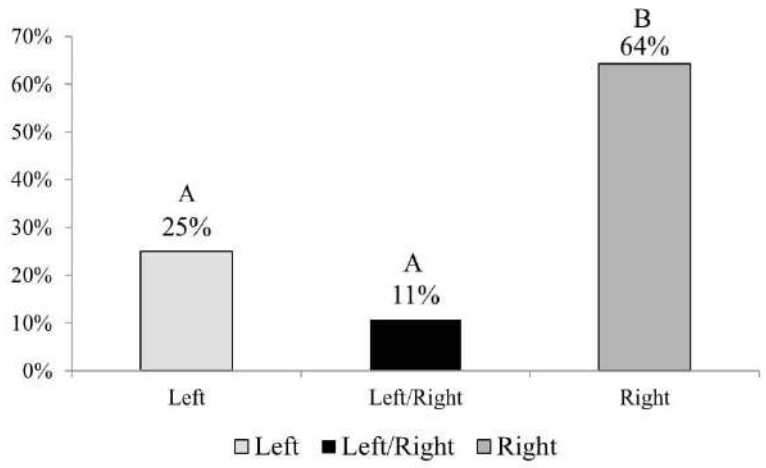

Figure 1. Percentage distribution of motor lateralization without taking into account gender of dogs $^{A, B}$ – The values marked with different letters within each breeding grounds differed significantly with $p \leq 0.01$(test for two structure indices).

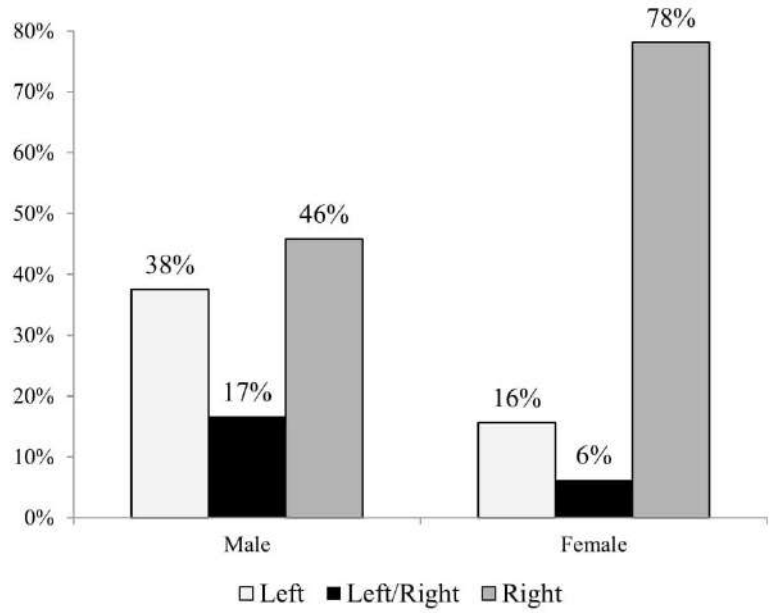

Figure 2. Percentage distribution of motor lateralization by gender of dogs (χ^2 = 6,238; df= 2; p = 0.0442).

The average cortisol level found in the blood of dogs with left-limb preference was (mean ± SE) 7.94 ± 0.81 μg/dl and was significantly higher than in the subjects from the other two groups. In individuals displaying right-sided lateralization the value was 3.92 ± 0.39 μg/dl and for ambilateral subjects 3.70 ± 0.54 μg/dl (Figure 3). When sex was taken into consideration, the mean cortisol concentration in female dogs' blood serum with left-sided lateralization was 7.17 ± 1.92 μg/dl and was higher than in female dogs with a preference for the right paw (3.37 ± 0.32 μg/dl), but the difference was not statistically significant. A tendency for higher cortisol levels in left-sided females was found. There were also no significant differences in cortisol levels between subjects with left paw preference and ambilateral female subjects (4.46 ± 0.99 μg/dl) (Figure 4). In a group of 24 male dogs, the average cortisol concentration in blood in subjects with left-sided lateralization was 8.37 ± 0.76 μg/dl and was significantly higher than in ambilateral (without preference) individuals (3.31 ± 0.65 μg/dl) (Figure 5). There were no significant differences between the ambilateral and the right-pawed individuals (5.16 ± 1.00 μg/dl).

The level of fT4 (thyroxine) was also examined in dogs. Higher thyroxine concentration was observed in subjects showing left-sided lateralization (mean ± SE) (1.95 ± 0.46 ng/dl) compared to dogs with right-sided lateralization (1.56 ± 0.23 ng/dl) and ambilateral (1.01 ± 0.22 ng/dl) (Figure 6). The differences were not statistically significant, and the level of thyroxine in blood serum in all study subjects was within the range of reference standards for healthy dogs 0.6–3.7 ng/dl (Winnicka, 2015).

Negative and highly significant correlation between cortisol concentration and thyroxine concentration in examined dogs was demonstrated (rs = −0.55; p = 0.0049).

Discussion

Many researchers point out the negative impact of stress on animal welfare during veterinary visits. In the current study, the levels of cortisol and thyroxine in blood serum were collected before a planned veterinary intervention during a visit to the veterinary surgery. The performed

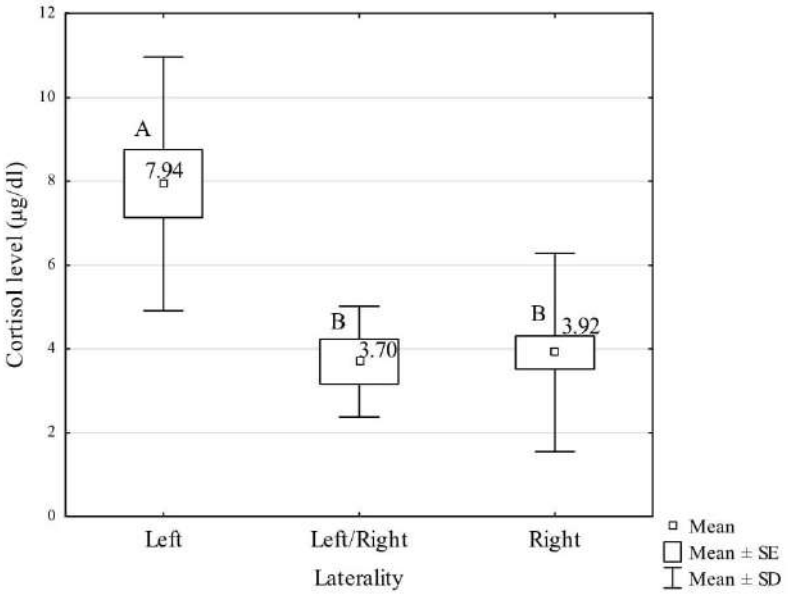

Figure 3. The concentration of cortisol in the blood of dogs (without gender division) depending on motor lateralization (ANOVA Kruskal-Wallis rank test, $\chi^2 = 13.730$; $p = 0.001$) $^{A, B}$ – The values marked with different letters within each breeding grounds differed significantly with $p \leq 0.01$.

medical and veterinary activities were treated as a potential source of stress. Cortisol levels are widely recognized as a good indicator of the body's current response in situations that include fear (Yuki et al., 2019). The study showed that the average cortisol level in all 56 tested animals was higher than the reference values in healthy dogs. Similar results were obtained by (Hennessy et al., 1997) who conducted a series of studies on human–dog interactions in an animal shelter. They found that during stress, cortisol levels in dogs increase approximately twice. Considering the blood cortisol reference values for dogs 1.0–6.8 µg/dl (Meyer & Harvey, 2004). the average cortisol level (7.94 µg/dl) obtained in our own study can be considered as high. This may indicate that exposure to a new place, new people, medical activities is undoubtedly a very stressful situation for dogs. Similar results were obtained by Hekman, Karas, and Dreschel (2012) who found an average level of cortisol of 8.7 µg/dl in dogs that were admitted to the veterinary clinics for scheduled procedures. Furthermore, cortisol levels were significantly higher in all animals tested. However, no significant differences in cortisol levels were seen in animals when their sex was taken into consideration. Similar results were obtained in the studies of Koyama, Omata, and Saito (2003), Reimers et al. (1990) and Sandri, Colussi, Perrotta, and Stefanon (2015) where no significant differences in cortisol levels in males and females were found.

In the current piece of research an attempt was made to estimate the relationship between the body's physiological response to stress (cortisol level) and motor lateralization of examined dogs. Having analyzed the relationship between motor lateralization found in dogs during the study and their cortisol concentration in plasma, it can be concluded that in the group of animals tested, individuals that showed the left paw preference, both in the group of females and males, were more susceptible to stress. Similar findings apply to research carried out by Rogers (2010) who found that animals with left-sided preference exhibit a higher level of stress than animals with right-sided lateralization that approached new objects with more confidence or did not overreact to new sounds, i.e., storm or fireworks. Branson (2006) came to similar conclusions, supporting the theory that

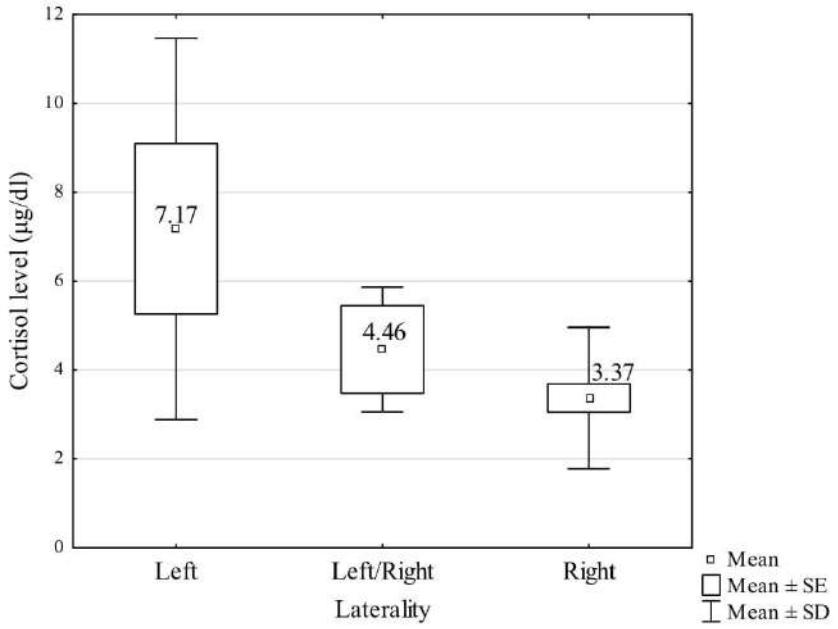

Figure 4. Cortisol concentration in bitches blood depending on motor lateralization (ANOVA Kruskal-Wallis rank test, $\chi^2 = 4.800$; $p = 0.091$).

lateralization of the brain modifies behavioral and physiological reactivity. The right cerebral hemisphere shows specialization in the context of the quality of responses resulting from the activation of the HPA axis. Dogs with the dominant right hemisphere demonstrate the preference for using the left paw and in the tests performed show a higher level of arousal compared to animals displaying the preference for the right paw in the test tasks (Westergaard et al., 2004). However, Batt et al. (2009) did not observe significant differences between cortisol levels and lateralization.

Pituitary gland cells are susceptible to cortisol, which inhibits the synthesis and release of TSH, resulting in a reduced concentration of thyroxine in plasma (Agha-Hosseini, Shirzad, & Moosavi, 2016). In the group of tested dogs, the fT4 value was determined. For the dogs that showed the left paw preference, the average was 1.95 ng/dl, 1.56 ng/dl was the average for the dogs with right paw preference and 1.01 ng/dl was the value for ambilateral dogs. According to (Meyer & Harvey, 2004). the obtained values are within the reference range of fT4 for healthy dogs which is 0.6–3.7 ng/dl. There was no significant relationship between cortisol and thyroxine in the blood serum.

Conclusion

The visit to the veterinary office as well as routine pre-surgical activities are a source of stress for many dogs, which was evidenced by the higher average cortisol level found in the blood serum of the study subjects compared to the reference cortisol level in healthy dogs. The average cortisol concentration significantly exceeded the reference values in all 56 examined dogs. The results obtained also seem to clearly indicate that dogs with left-sided motor lateralization are much more susceptible to stress. In the examined group of dogs, both females and males preferring to use the left paw in the test were characterized by higher average cortisol concentration in the blood serum. The results of fT4 concentration obtained in our own research that fall within the reference standards range seem to indicate the lack of explicit links between thyroid endocrine activity and

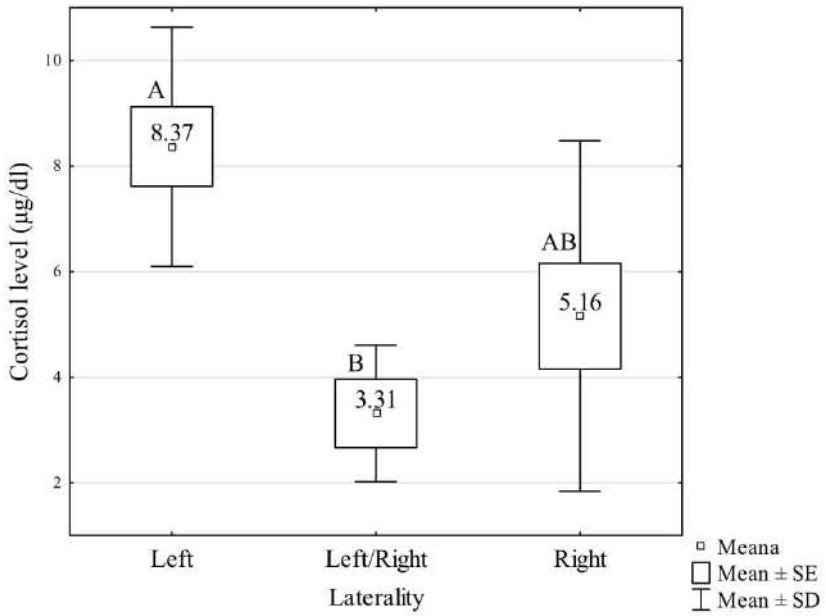

Figure 5. Cortisol concentration in male blood depending on motor lateralization (ANOVA Kruskal-Wallis rank test, $\chi^2 = 10.263$; $p = 0.006$) $^{A, B}$ – The values marked with different letters within each breeding grounds differed significantly with $p \leq 0.01$.

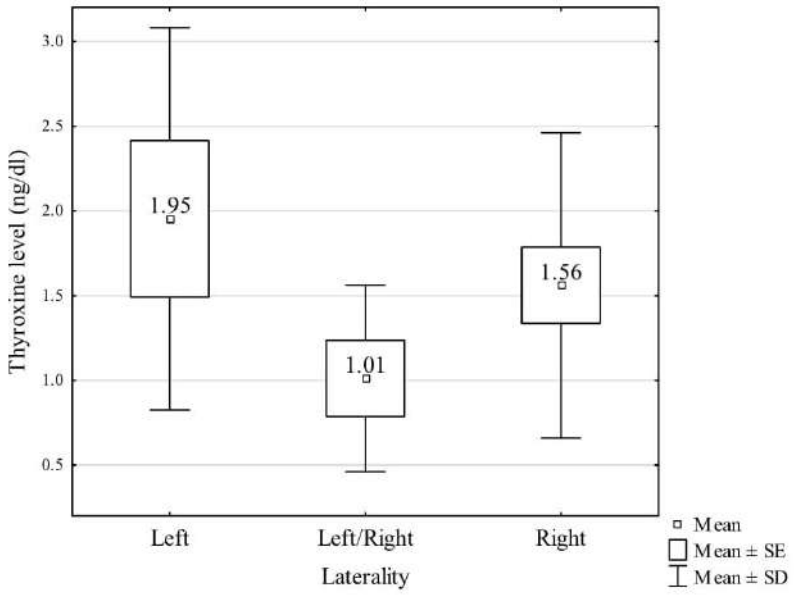

Figure 6. Thyroxine (fT4) concentration in dogs' blood (without gender) depending on motor lateralization (ANOVA Kruskal-Wallis rank test, $\chi^2 = 0.917$; $p = 0.632$).

stimulation resulting from the procedures performed during a visit to the veterinary clinic. The obtained results can be used for a quick assessment of dogs susceptibility to stress (based on the preferred limb) and, as a result, individualized actions in the context of its mitigation. Testing for paw preferences can also be a useful tool to apply appropriate strategies when dealing with dogs in a variety of situations to reduce the risk of causing a decrease in welfare.

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MDPI

Communication

Temperament Assessment Algorithm in Dogs

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Simple Summary: The assessment of the personality and temperament of an animal is becoming more popular and appreciated not only by the keepers of working dogs, but also by the owners of companion dogs. The aim of our work was to create a short questionnaire in the form of a table for animal keepers with 24 specific temperament traits listed. Two veterinarians and forty-six animal behaviorists (owners of the observed dogs) participated in the study by observing the behavior of dogs in their natural environment. On the basis of the selected features, the temperament of the dogs was determined and assigned to one of two groups: introverts or extroverts.

Abstract: The aim of this study was to evaluate the temperament of dogs on the basis of behavioral observations, with emphasis on 24 selected traits and behaviors. From the observations, the temperament of the dogs was determined and the animals were assigned to one of two personality groups: introvert or extrovert. The study involved 46 dogs. The agglomeration method, Pearson's 1-*r* distance, and Ward's binding method were used. As shown by the statistical analysis, 18 dogs (39%) were assessed as introverts and 28 dogs (61%) exhibited extrovert traits. To construct a model for the assessment of canine temperament using the identified traits, logistic regression was performed with the independent variables, number of extrovert traits (ETs) and introvert traits (ITs), and a dichotomous dependent variable (1 = extrovert, 0 = introvert), reflecting the assessment of the temperament of the dog based on the observations and results of the original questionnaire.

Keywords: dog; temperament; personality; extrovert; introvert

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1. Introduction

Personality and temperament are terms used in psychology to describe different ways of feeling and behaving. Temperament is an essential part of personality [1]. There are two basic terms in the ethological literature, i.e., temperament and personality. Personality is understood as a set of psychological traits that determine the reaction of an animal to a given situation [2]. Temperament, in turn, is understood as an innate tendency to display certain traits [1]. Temperament is defined as the biological and instinctive part of personality. In fact, this part of personality always shows first. The first discoveries in this field were made by Ivan Pavlov, who studied dog conditioning to associate a specific sequence of events with stimuli [3]. Since it is primarily determined by innate physiological mechanisms, changes in temperament are induced by puberty, aging, and environmental factors. Approximately 40% of temperament traits have a genetic (hereditary) background, whereas 50–60% of these traits are determined environmentally. Hence, the same dog examined at the age of 1 year and again at the age of 10 years may turn out to have the same temperament traits, although manifested differently. Temperament cannot be evaluated as being either good or bad; it is relatively constant, but manifests itself in variable behaviors [4]. It influences the development of personality and its traits. A very strong impact of temperament has been detected in puppies. At the stage of adaptation to the environment during further development, its influence on behavior weakens and the impact of the experience gained becomes important.

The most common method for assessment of human personality is the Eysenck Personality Questionnaire-Revised [5]. In accordance with the personality theory proposed by Costa and McCrae [6], the assessment is based on one of the five factors of the personality model, i.e., extroversion. The so-called "big five" personality traits should be regarded as a tendency to behave in a certain way in a situation that is conducive to its expression. For example, a dog may be both an extrovert, with readiness to retrieve and jump in a play-provoking situation, and an introvert, guarding resources and its individual comfort zone in the household. This does not mean a change in the trait; the change lies in the situation (expression) in which the dog exhibits introverted behavior despite its extroversion, or vice versa. There is no contradiction in this claim. In the DPQ (dog personality questionnaire) personality test battery, the authors assessed dog temperament traits (e.g., self-confidence, aggression, excitability, self-control, distance, submissiveness, and assertiveness) in different situations. These included walking on a leash with a familiar person/stranger, a friendly/threatening situation, being stroked by a familiar person/stranger, being grabbed by the neck, and contact with a novel object, etc. [7].

Observations of the behavior of a given animal allow the assignment of a specific personality trait, as in the present study. Dog personality traits such as boldness, exploration, aggression, activity, and social tendency are assessed most frequently [8]. Although animals cannot answer the study questions, researchers use a questionnaire method to evaluate the temperament traits of dogs based on responses obtained from their owners [9]. Since keepers know their pets best and observe them throughout their lives in a variety of situations, it is believed that their answers to the questions can be considered a reliable source of information about the personality of the dog, as demonstrated by Gosling [10]. The most popular questionnaire for the assessment of canine personality is Serpell's Canine Behavioral Assessment and Research Questionnaire (C-BARQ), which contains 101 questions [11]. Considerable agreement of results has been shown between the C-BARQ test and DMA (Dog Mentality Assessment) behavioral test [12]. An increasing number of scientists investigating dog personality traits use questionnaires/surveys addressed to the animal keeper, and these exhibit great efficiency in the determination of personality types.

One of the many theories of personality assessment is Eysenck's theory, which is based on division into introverted and extroverted personality types. A study conducted by Gosling and John [2] showed similarities in specific personality traits between humans and dogs. However, there is no well-validated, reliable, and effective instrument for dog personality assessment characterized by easy and quick use and a wide range of applications.

The aim of the study presented herein was the development of a shortened questionnaire that would be understandable and simple to an animal owner who is not an animal behaviorist. The questionnaire was created with the involvement of veterinarians (supervisors–originators) and animal behaviorists (owners of the tested dogs). However, it was targeted at all dog owners to help them understand the dog's type of temperament in a clear and friendly manner.

An additional application value of the developed algorithm is that it can be used during a short visit to the veterinary practice, while admitting the dog to a hotel, or during behavioral consultations. There are many scientific papers reporting that the determination of dog's temperament takes several days and is a very complex process. The authors of this study aimed to produce a completely different/practical assessment of temperament.

2. Materials and Methods

The study involved 46 dogs and their keepers (certified animal behaviorists) whose task was to indicate the presence or absence of the analyzed traits in the dogs in the designed questionnaire (Table 1). The test leaders (two veterinarians) were responsible for guiding the owners to fill in the test correctly. The dogs were observed and evaluated under neutral environmental conditions without stressful or distracting stimuli. All dogs were domestic companion dogs who did not show extreme features, such as excessive

aggression or excessive timidity, and extreme expression in an animal with a balanced character was noted during the behavioral observations. They were also clinically healthy and neutered. The authors deliberately did not take into account such parameters as sex, age, breed, or type of maintenance in the subsequent analyses.

Table 1. Authors' questionnaire with the 24 selected temperamental 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	

The traits shown in the table were selected from many scientific publications [7,12–14] in order to choose only typical extroverted and introverted temperamental traits and to minimize the time and resources required for assessment of the temperament of the dogs. Statistical analysis of the results was conducted using the agglomeration method, Pearson's 1-*r* distance, and Ward's binding method.

3. Results

In the study group, the temperament of 18 dogs (39%) was assessed as introverted, while 28 dogs (61%) exhibited an extroverted temperament (Figure 1 and Table 2). Moreover, two clusters of the analyzed traits visible in the hierarchical tree diagram were detected (Figure 2). 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. Notably, the traits from cluster 1 were assigned to individuals with an extroverted temperament, while the traits from cluster 2 are typical of introverted animals. This study thus confirms the same grouping of temperament traits in dogs.

Table 2. Distribution of temperament traits in both subject groups (*n*, % in the group).

Trait	Introvert	Extrovert	Total
Wise	9 50.00%	22 78.57%	31 67.39%
Sociable	1 5.56%	28 100.00%	29 63.04%
Curious	0 0.00%	22 78.57%	22 47.83%
Cheerful	1 5.56%	20 71.43%	21 45.65%
Dominant	2 11.76%	8 28.57%	10 21.74%
Noisy	5 27.78%	25 89.29%	30 65.22%
Disobedient	8 44.44%	22 78.57%	30 65.22%
Hyperactive	8 44.44%	22 78.57%	30 65.22%
Clever	2 11.11%	13 46.43%	15 32.61%
Impatient	7 38.89%	23 82.14%	30 65.22%
Bold	1 5.56%	13 46.43%	14 30.43%
Territorial	3 16.67%	23 82.14%	26 56.52%
Stubborn	16 88.89%	12 42.86%	28 60.87%
Loner	18 100.00%	0 0.00%	18 39.13%
Aggressive	6 33.33%	11 39.29%	17 36.96%
Lazy	15 83.33%	10 35.71%	25 54.35%
Greedy	17 94.44%	17 60.71%	34 73.91%
Alert	9 50.00%	11 39.29%	20 43.48%
Unpredictable	17 94.44%	5 17.86%	22 47.83%
Fearful	17 94.44%	0 0.00%	17 36.96%
Distrustful	18 100.00%	5 17.86%	23 50.00%
Insecure	16 88.89%	4 14.29%	20 43.48%
Absent-minded	11 61.11%	3 10.71%	14 30.43%
Timid	17 94.44%	0 0.00%	17 36.96%

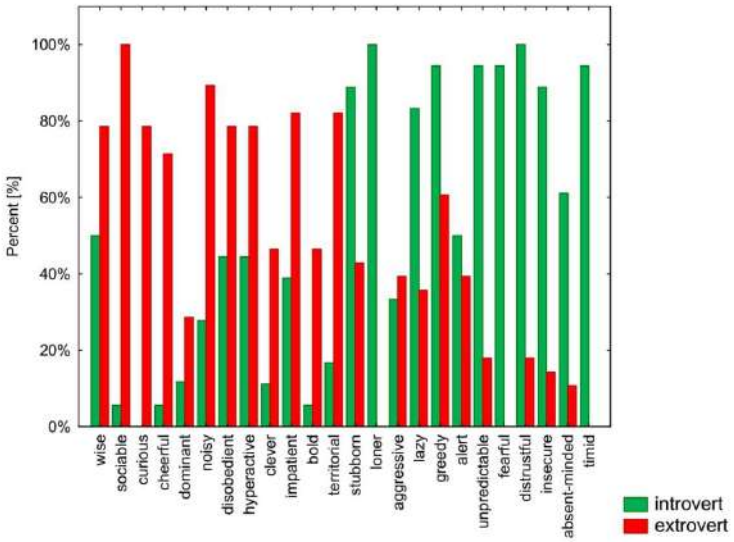

Figure 1. Percentage distribution of the observed traits in both subject groups.

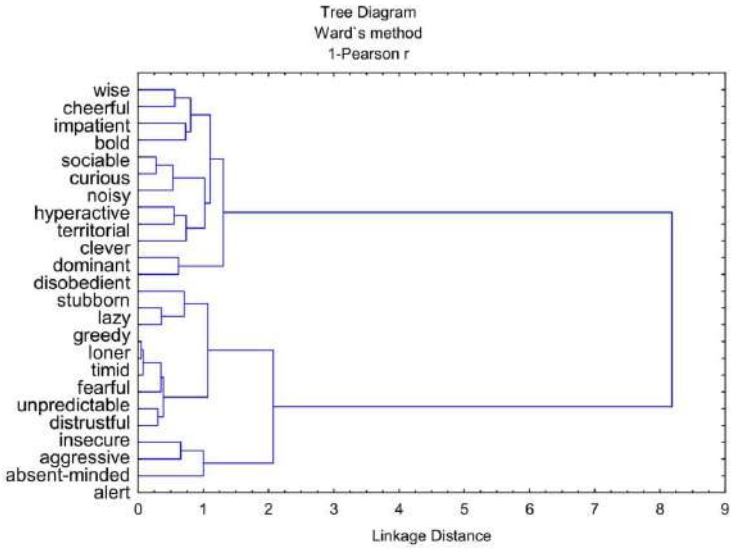

Figure 2. Hierarchical tree chart with two clusters of features.

The number of ETs (in cluster 1) and the number of ITs (in cluster 2) indicated in the questionnaire of each dog were counted. In order to construct a model for the assessment of the temperament of the dogs based on the survey results, a logistic regression was performed with the independent variables ETs and ITs and a dichotomous dependent variable Y (1 = extrovert, 0 = introvert), indicating the temperament based on the observations of the behaviorist.

A logistic regression model with parameters presented in the table below (Table 3) was developed:

$$P\,(Y = 1|ET = x, IT = y) = \frac{\exp(a_0 + a_1 x + a_2 y)}{1 + \exp(a_0 + a_1 x + a_2 y)}, \quad (1)$$

where $P\,(Y = 1|ET = x, IT = y)$ is the probability that the dog is extroverted if $ET = x$ and $IT = y$.

Table 3. Logistic model parameters.

Parameter	a_0	a_1	a_2
Rating	−10.92	3.99	−2.39

In the logistic regression model, the substitution of the number of extrovert and introvert traits, as well as model parameters, to the right side of the equation for each analyzed dog yielded the value of the probability that the dog has an extroverted personality. For the probability values of $p > 0.5$ and $p < 0.5$, the dogs were classified as extroverts or introverts, respectively. Thus, based on the questionnaire, the model classified the dogs into appropriate thematic groups (Figure 3).

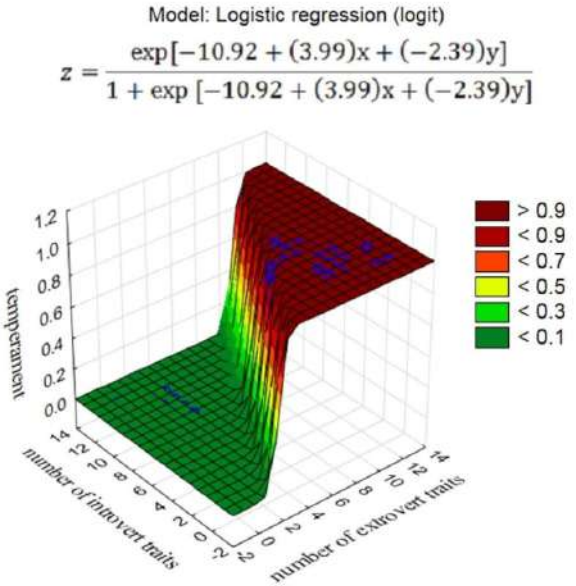

Figure 3. Logistic regression model.

The presented model exhibited 100% accuracy in the classification of the dogs with both extroverted and introverted temperaments. Certainly, it should be borne in mind that these were post hoc classifications, as the calculation of parameters targeted the minimization of the probability of the observed data. Therefore, the current model used for classification of new observations in the future may have a slightly lower accuracy (Table 4).

Table 4. Classification of logistic model cases.

Observed	Predicted Introvert	Predicted Extrovert	Percentage
Introvert	18	0	100.00
Extrovert	0	28	100.00

4. Discussion

For many years, scientists have been trying to match known human and animal personality models to animals [15]. The scientific literature distinguishes four main methods for the assessment of dog behavior, i.e., test batteries, individual assessments of dogs using a questionnaire, observations conducted under natural conditions, and expert assessments of individual breeds [16]. Other approaches for personality assessment include coding and evaluation methods. Coding involves the observation and interpretation of the behavior of the animal in a specific situation, and estimation is based on subjective attribution of a given trait [17], similar to this work.

Surveys involving owners or handlers of animals for the assessment of the temperament or behavior of said animals were introduced in the last century and are still widely used and accepted in animal personality studies [18]. Hsu and Serpell [14], Jones and Gosling [1], Diederich and Giffroy [16], and Taylor and Mills [19] reviewed the majority of the available literature reports on temperament testing, including a meta-analysis of results and methods.

The division into extroverted and introverted personality types is one of the most accurately and easily identified dimensions of human personality [20]. Analogies between human and dog personality traits have been reported by some scientists [21]. The personality types identified by the authors based on the survey results can be considered analogous to human extroversion or introversion, which is corroborated by the selection of the adjectives compiled in the table. This is a common finding in studies on many other animal species [2], including cats [22] and horses [23]. A similar division into individual personality components in chimpanzees was presented by King and Figueredo [24]. Moreover, a questionnaire-based personality assessment was performed in a group of guide dogs [14], which may emphasize its effectiveness and suitability.

Previous questionnaire-based assessments of personality or temperament have covered a very wide range of individual traits and required substantial work, e.g., over 100 questions in the study conducted by Clay et al. [11] or 152 questions in that by Hsu and Serpell [14]. Other studies, e.g., De Meester et al. [25], have focused on the assessment of only some temperamental traits, such as self-confidence, submissiveness, or timidity, without conclusively specifying the personality of the tested animals. Other investigations have consisted of asking the owner extensive questions (LAPS), which may be confusing and the answers may be puzzling; often, they do not satisfactorily determine the character of the animal [26]. In practice, the use of these methods is difficult and is sometimes even impossible due to inappropriate conditions and the owner's weariness with the scale of difficulty. The questionnaire designed in the present study can be helpful for quick personality assessment, e.g., during a visit to a veterinary clinic or during behavioral consultation.

Although satisfactory research results have been reported, a large number of studies based on the questionnaire method require the standardization of the adjectives that describe personality and a clear division of personality based on the assessed traits. The authors of the present study hope that their method will be useful in the discrimination between extroverted and introverted personality types in dogs. Future research is planned to include the impact of sex, breed, and age on the personality of a dog. The development of tests that are not based solely on the subjective assessment of a dog's behavior will also be investigated. The authors plan to perform short tests after subjective estimation of the temperament on the present algorithm by the caregiver, which will confirm or exclude the presence of the features marked in the questionnaire. In addition, it is planned to study

a larger group of dogs and their owners in order to estimate the relationship between temperament and independent characteristics, such as gender, age, color, and breed.

5. Conclusions

To sum up, personality is regarded as a set of mental traits determining an animal's reaction to a given situation. However, despite the many studies performed, there is still no reliable method for the clear assessment of dog personality. Many researchers are developing innovative methods to estimate animals' character traits reliably. The knowledge of the personality traits of dogs is helpful in the selection of an animal for a specific role, e.g., as a guide, rescuer, or therapist. Improving the effectiveness of the adoption of shelter dogs is becoming increasingly important as well [12]. Personality tests used to assess whether a puppy will be a suitable companion for the future owner are becoming more popular [27].

To address the complexity of tests for assessing canine personality, the authors of this paper created a short questionnaire to estimate temperament as a part of canine personality. The authors of this paper were able to create this questionnaire with the help of qualified animal behaviorists and veterinarians. It is aimed at people/owners of dogs who are not familiar with animal behavior, and provides an estimate of the tendency of temperament toward extroversion or introversion based on their own observations. With the above pioneering research, any pet owner or animal caretaker at a shelter or animal hotel can estimate the temperament of a pet in a short period of time.

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