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VARIABILIDADE DA FREQUÊNCIA CARDÍACA, ESTRESSE OXIDATIVO E CARACTERIZAÇÃO DO SONO EM PERÍODO NOTURNO DE CÃES BRAQUICEFÁLICOS E MESOCEFÁLICOS EM AMBIENTES DOMICILIAR E HOSPITALAR

REALEZA
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Dissertação apresentada ao Programa de Pós-Graduação em Saúde, Bem-Estar e Produção Animal Sustentável na Fronteira Sul da Universidade Federal da Fronteira Sul (UFFS), como requisito para obtenção do título de Mestre em Saúde, Bem-Estar e Produção Animal Sustentável na Fronteira Sul.

Orientador: Prof. ª Dr. ª Tatiana Champion

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RESUMO

O ambiente é um fator crucial que pode modificar as dinâmicas do sono, em humanos, é bem documentada a má qualidade do sono em pacientes hospitalizados, caracterizada por menos tempo de sono e qualidade reduzida e em cães, o ambiente também interfere significativamente no sono, reduzindo consideravelmente o período de descanso desses animais. Além das disritmias causadas pelo ambiente, raças braquicefálicas são particularmente predispostas a desenvolver distúrbios do sono, como a apneia, devido à obstrução parcial das vias aéreas, comum nessas raças. Tanto o ambiente quanto os distúrbios do sono afetam os mecanismos fisiológicos dos cães, a variabilidade da frequência cardíaca (VFC) é uma ferramenta importante para avaliar o balanço autonômico através das flutuações na frequência cardíaca, sendo um indicador de situações estressantes. O estresse oxidativo resulta do desbalanço entre espécies reativas de oxigênio e os mecanismos antioxidantes do organismo, podendo ser desencadeado por situações de estresse, como o ambiente hospitalar e os distúrbios do sono. Com base nestas premissas, esta dissertação foi organizada em dois capítulos, apresentados como artigos científicos. O primeiro artigo aborda a variabilidade da frequência cardíaca e a caracterização do sono de cães braquicefálicos e mesocefálicos em ambientes domiciliares e hospitalares. O segundo artigo examina o estresse oxidativo em cães braquicefálicos e mesocefálicos nesses mesmos ambientes. Por meio dos estudos foi possível evidenciar a ausência do estímulo vagal em cães braquicefálicos, que o ambiente hospitalar tem efeito na geração de estresse oxidativo em cães braquicefálicos e mesocefálicos, que a conformação racial e o ambiente não exercem efeito no tempo de sono noturno ao comparar cães braquicefálicos e mesocefálicos em ambiente domiciliar e hospitalar e que o ambiente hospitalar não tem efeito sobre a variabilidade da frequência cardíaca de cães braquicefálicos e mesocefálicos.

Palavras-chave: Equilíbrio autonômico; Hospitalização; Estresse Oxidativo.

ABSTRACT

The environment is a crucial factor that can modify sleep dynamics. In humans, poor sleep quality in hospitalized patients is well documented, characterized by less sleep time and reduced quality. In dogs, the environment also significantly interferes with sleep, considerably reducing their rest period. Besides the arrhythmias caused by the environment, brachycephalic breeds are particularly predisposed to developing sleep disorders, such as apnea, due to partial airway obstruction common in these breeds. Both the environment and sleep disorders affect the physiological mechanisms of dogs. Heart rate variability (HRV) is an important tool for evaluating autonomic balance through heart rate fluctuations, serving as an indicator of stressful situations. Oxidative stress results from the imbalance between reactive oxygen species and the body's antioxidant mechanisms, which can be triggered by stressors such as the hospital environment and sleep disorders. Based on these premises, this dissertation is organized into two chapters, presented as scientific articles. The first article addresses heart rate variability and sleep characterization in brachycephalic and mesocephalic dogs in home and hospital environments. The second article examines oxidative stress in brachycephalic and mesocephalic dogs in these same environments. Through the studies, it was possible to evidence the absence of vagal stimulation in brachycephalic dogs, that the hospital environment has an effect on the generation of oxidative stress in both brachycephalic and mesocephalic dogs, that racial conformation and the environment do not affect nighttime sleep duration when comparing brachycephalic and mesocephalic dogs in home and hospital environments, and that the hospital environment does not affect heart rate variability in brachycephalic and mesocephalic dogs.

Keywords: Autonomic balance; Hospitalization; Oxidative Stress.

SUMÁRIO

1 INTRODUÇÃO

1.1 SONO DOS CÃES

O sono é definido como um estado alterado de consciência de ocorrência natural e periódica, caracterizado por inatividade motora, estado de consciência e resposta a estímulos ambientais reduzidos ao mínimo. No entanto, pode ser revertido rapidamente a depender de estímulos específicos, que o distingue de estados de consciência alterados como anestesia e o coma (GREENE; SIEGEL, 2004; MAVANJI et al., 2012). Em média, o ser humano dorme de sete a oito horas diárias, em comparação o cão dorme 10 horas, podendo variar de sete a 16 horas, no entanto, cães apresentam uma fase de sonolência, considerada como um sono leve, que representa 21 % do tempo total de sono (BÓDIZS et al., 2020; KNUTSON, 2010; TOOLEY; HEATH, 2022).

Diversas funções do organismo, como controle homeostático, estão relacionadas com o sono, tornando-o de extrema importância. As variações de temperatura corporal encontradas em cães indicam um padrão de vigília-sono como diurno, o período de sono predomina durante a noite sendo mais efetivo nesse período, no entanto podem ocorrer momentos de sono durante o dia (BÓDIZS et al., 2020; KINSMAN et al., 2020; LUCAS; POWELL; MURPHREE, 1977). Ademais, os cães possuem uma ausência variação de cortisol que sugere uma regulação circadiana mais fraca do que a encontrada em seres humanos, resultando em uma maior flexibilidade em tempo de sono e uma fácil adaptação a mudanças de rotina (BÓDIZS et al., 2020).

O sono dos cães é caracterizado como polifásico, ocorrendo principalmente entre às 21:00 e as 6:00 horas. O sono pode ser classificado simplificadamente em dois níveis, sono REM (rapid eye movement) e sono NREM (non-rapid eye movement) (MAVANJI et al., 2012; RIAL et al., 2023). Por meio de estudos realizados em humanos, o sono NREM é classificado em estágios, diferentes padrões são observados na eletroencefalografia, sendo dividido em N1, N2 e sono de ondas lentas (SWS). Já segundo a literatura veterinária as definições de SWS e NREM são utilizadas com o mesmo objetivo e normalmente referindo-se ao estágio do sono que representa o NREM em cães (MAVANJI et al., 2012).

O sono pode ser identificado de diversas formas, como: eletroencefalografia, atividade muscular, movimento dos olhos conjuntamente com outros sinais como frequência cardíaca e respiratória e temperatura corporal (RIAL et al., 2023). Em cães o sono pode ser avaliado por critérios comportamentais e critérios poligráficos. Os critérios comportamentais são inatividade

motora, estereótipos de postura, limiares sensoriais aumentados, reversibilidade e capacidade de despertar, regulação homeostática e organização do ciclo circadiano e fechamento dos olhos. Estes critérios podem ser avaliados por gravação de vídeos, eletroencefalograma e polissonografia, e actigrafia. Os critérios poligráficos são o eletroencefalograma, eletro-oculograma e eletromiograma para identificação do sono REM e NREM que são avaliados por meio de eletroencefalograma e polissonografia invasiva (BÓDIZS et al., 2020).

Diferentes funções são atribuídas as fases do sono, é observado que durante o NREM ocorrem processos de recuperação do sistema nervoso, defesa contra estresse oxidativo e conservação de energia (ATHANASOULI et al., 2023; MAVANJI et al., 2012). Um sono ideal tem início no NREM e é seguido pelo sono REM, que é associado com as funções de consolidação de memória, crescimento, modulação da atividade neural, neurogênese e desenvolvimento do sistema nervoso (MAVANJI et al., 2012; MUKAI; YAMANAKA, 2023). A fase REM do sono ainda pode ser dividida em duas instâncias, REM fásico e REM tônico, sendo que espasmos musculares, movimento dos olhos e vocalizações ocorrem durante o período fásico do sono (RIAL et al., 2023).

1.2 SÍNDROME BRAQUICEFÁLICA

A Síndrome Braquicefálica é uma causa comum de angústia respiratória devido a alteração de componentes anatômicos primários e sequelas secundárias. As alterações anatômicas compreendem a narinas estenosadas, palato mole alongado, traqueia hipoplásica, macroglossia, eversão de sacos laríngeos, colapso traqueal e colapso laríngeo, que podem representar complicações respiratórias severas (DUPRÉ; HEIDENREICH, 2016; MEOLA, 2013).

Comum à Síndrome Braquicefálica, cães apresentam um aumento de comprimento e espessura de língua, denominado como macroglossia, que contribui com um deslocamento dorsal do palato mole, reduzindo o espaço da nasofaringe. A alteração do volume da língua está relacionada com o braquignatismo maxilar profundo encontrado em cães braquicefálicos. O volume relativo da língua de cães braquicefálicos é aumentado em relação a de cães mesocefálicos, no entanto o volume tem variação entre raças braquicefálicas. Buldogue-Inglês e Buldogue-Francês possuem um volume de língua aumentado em comparação com Pugs (JONES; STANLEY; NELSON, 2020; SIEDENBURG; DUPRÉ, 2021).

As raças consideradas braquicefálicas são Pugs, Buldogue-Inglês, Buldogue-Francês, Boston Terrier, Pequinês, Maltês, Shih Tzu, Boxers, Cavalier King Charles Spaniel, Yorkshire

Terrier, Chihuahuas, Dogue-de-Bordéus e Bullmastiff (DUPRÉ; HEIDENREICH, 2016; MEOLA, 2013). Cães braquicefálicos necessitam superar uma resistência respiratória aumentada, tornando a expiração um ato forçado. A resistência respiratória pode chegar a 16 vezes mais do que outras raças e a força necessária para superar a resistência cria um aumento da pressão negativa, resultando em inflamação de tecidos moles, eversão de sacos laríngeos e tonsilas, e ainda, colapso traqueal e laríngeo (DUPRÉ; HEIDENREICH, 2016; MEOLA, 2013; PHILLIPS, 2022). Os cães que possuem essa condição normalmente apresentam sinais clínicos respiratórios típicos como estertor, estridor, ronco, tosse, intolerância ao exercício, esforço respiratório aumentado, hipertermia, cianose e em casos mais graves episódios de síncope. A síndrome braquicefálica é uma enfermidade progressiva, os sinais clínicos podem variar de uma gravidade mínima até crises respiratórias graves devido a colapso traqueal e laríngeo (DUPRÉ; HEIDENREICH, 2016; MEOLA, 2013; PHILLIPS, 2022).

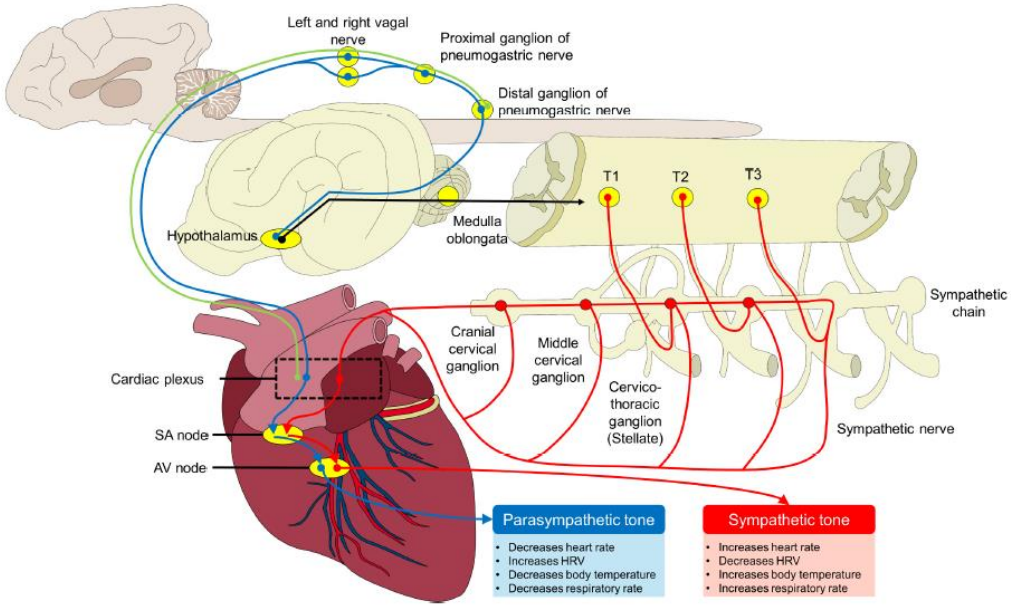
As síndromes respiratórias do sono são comumente observadas em cães braquicefálicos. Dificuldade respiratória no período de vigília e hipersonolência são sinais que indicam a presença de alguma condição respiratória durante o sono (BARKER et al., 2021; MEOLA, 2013). O espectro dos distúrbios respiratórios do sono é extenso, a síndrome da apneia obstrutiva do sono (SAOS) está localizada no extremo do espectro devido a elevado grau de severidade, principalmente em cães braquicefálicos que apresentam características anatômicas como narinas estreitas, turbinados nasais tortuosos e palato mole deslocado caudalmente, que contribuem na gravidade do distúrbio (HENDRICKS et al., 1987; NOCERA; CONNOLLY, 2023; SHETTY et al., 2023). Durante o sono o relaxamento muscular pode resultar em colapso do palato mole e bloqueio das vias aéreas, podem levar a uma obstrução parcial do fluxo respiratório ou uma pausa completa da respiração. Esses eventos levam a uma redução da saturação do oxigênio sanguíneo e a uma interrupção da continuidade do sono (HENDRICKS et al., 1987; NOCERA; CONNOLLY, 2023).

Os cães da raça Buldogue-Inglês são modelos naturais de distúrbios respiratórios do sono. Respiração anormal durante o sono, com piora durante o sono REM, respiração desordenada com apneia obstrutiva central, episódios de dessaturação de oxigênio e hipersonolência são os principais eventos apresentados pela espécie. Os distúrbios respiratórios são piores durante o sono REM, isso ocorre devido a supressão da atividade muscular esquelética que ocorre durante o sono REM, suprimindo grupos musculares da porção superior respiratória e músculos respiratórios do tórax. A redução da atividade muscular resulta na dilatação dos músculos laríngeos e faríngeos, aumenta a resistência da passagem de oxigênio e contribui para um colapso das vias aéreas superiores (HENDRICKS et al., 1987).

1.3 VARIABILIDADE DA FREQUÊNCIA CARDIACA

A análise da variabilidade da frequência cardíaca é um método não invasivo para avaliação do equilíbrio do sistema nervoso autônomo. Por meio de análises de flutuações instantâneas na frequência cardíaca, o método, estima a regulação autonômica (BIDOLI; ERHARD; DÖRING, 2022; VON BORELL et al., 2007). A variabilidade da frequência cardíaca (VFC) inicialmente surge por meio da atividade de ramos do sistema nervoso autônomo, que é influenciado por mecanismos neurais, humorais e fisiológicos (Figura 1) (VON BORELL et al., 2007).

Figura 1 - Representação visual do controle autonômico cardiovascular. Ramos nervosos parassimpáticos (linhas azuis) e simpáticos (linhas vermelhas) controlam as respostas dos animais ao estresse. Alterações controladas pelo sistema simpático, que envolvem taquicardia, taquipneia ou hipertermia, são desencadeados pelos nervos pré e pós ganglionares (linhas pretas) e por estímulos de cadeia simpática. Já, diminuição da frequência cardíaca, na temperatura corporal, na frequência respiratória e aumento da VFC estão relacionadas ao sistema parassimpático, mediado por neurônio dos gânglios cardíacos, dependendo da regulação do nervo vago no nodo sinoatrial e atrioventricular (GHEZZI et al., 2024).



(GHEZZI et al., 2024)

A VFC no domínio do tempo é a flutuação dos intervalos entre ciclos cardíacos adjacentes. Seus índices envolvem diversas funções neuro-cardíacas e suas interações, apesar da forte relação com a frequência cardíaca, a VFC reflete a regulação do balanço autonômico, pressão arterial, trocas de gases e tônus vascular (SHAFFER; GINSBERG, 2017). Os índices do balanço autonômico podem ser obtidos por diferentes técnicas, sendo categorizadas principalmente em domínio temporal e domínio de frequência (ZUPAN et al., 2016). A análise do domínio da frequência transforma a variabilidade dos intervalos entre ciclos cardíacos em

dois tipos de bandas de frequência. As ondas de frequência alta representam marcadores de atividade parassimpática, já as ondas de frequência baixa representam marcadores de atividade simpática e parassimpática (BIDOLI; ERHARD; DÖRING, 2022). O domínio do tempo envolve, principalmente, um índice obtido por meio da raiz quadrada da média de intervalos entre ciclos sucessivos, dando origem ao RMSSD (root mean square of successive differences between normal heartbeats) um dos principais indicadores de variações relacionadas à ativação parassimpática e desvio padrão de intervalos normais a normais, dando origem ao SDNN (standard deviation of the interbeat interval of normal sinus beats), um índice utilizado como prognóstico de risco cardiovascular em humanos (QIN et al., 2021; ZUPAN et al., 2016).

A frequência cardíaca pode ser um indicador do estado fisiológico em animais e em humanos, normalmente associado a níveis altos de estresse, tornando-a indicador fisiológico de regulação das emoções, devido às inervações autonômicas do coração (VARGA et al., 2018).

1.4 ESTRESSE OXIDATIVO

Radicais livres são moléculas que possuem um ou mais elétrons despareados em sua órbita externa, propícios a reagir molecularmente. Dessa forma, quando um radical reage com uma molécula não radical, ocorre uma transferência de elétrons de uma molécula para outra através de reações redução-oxidação (redox), propagando uma cadeia de reações (LAVIE, 2015).

Como os radicais livres são subprodutos do metabolismo normal do oxigênio, gerados na respiração celular, um sistema enzimático e não enzimático antioxidante tem o papel de eliminar o excesso destes radicais. Quando a geração de espécies reativas ao oxigênio (EROS) excede a capacidade antioxidante celular, estresse oxidativo e dano celular e tecidual pode acontecer. A mitocôndria é a fonte principal de radicais durante a respiração celular e durante a hipóxia, a concentração de oxigênio é reduzida e a produção de radicais é aumentada devido à redução excessiva da ativação mitocondrial. A principal fonte de criação de radicais refere-se a hipóxia e reperfusão, a constante hipóxia seguida de reperfusão dos tecidos induzem alterações no metabolismo de energia e alteração de expressão de genes (LAVIE, 2003).

A atividade da enzima antioxidante superóxido dismutase (SOD) é reduzida em cães braquicefálicos. A baixa atividade da enzima pode ser explicada pelo efeito de hipóxia-reperfusão causado pela obstrução das vias aéreas, que pode ocasionar distúrbios de sono relacionados a apneia e hipoxemia. São evidenciados níveis menores que 90% de saturação de oxigênio em longos períodos durante o sono e momentos de apneia em cães da raça Buldogue-

Inglês. Cães braquicefálicos apresentam episódios de apneia semelhantes a seres humanos que possuem Síndrome da Apneia do Sono. Esses eventos resultam em um aumento da geração de EROS e espécies reativas de nitrogênio, que podem levar a disfunção endotelial, inflamação vascular, aterosclerose e papel principal no desenvolvimento de diversas doenças cardíacas (ERJAVEC; VOVK; SVETE, 2021).

Em seres humanos, episódios recorrentes de distúrbio do fluxo aéreo, causados pela obstrução durante o sono podem levar a apneia e flutuação da concentração de oxigênio no sangue, essa constante hipoxemia e reoxigenação resultam em um aumento da produção de EROS levando ao estresse oxidativo sistêmico, produção de fatores pró inflamatórios, desregulação metabólica e agregação plaquetária. O estresse oxidativo sistêmico pode representar um papel principal na disfunção endotelial e ser uma peça principal no aumento de risco cardiovascular de seres humanos acometidos com distúrbios respiratórios do sono (LAVALLE et al., 2024; MASTINO et al., 2023; STANEK; BROŻYNA-TKACZYK; MYŚLIŃSKI, 2021; YAMAUCHI et al., 2005).

Com base no apresentado acima, essa dissertação teve como objetivo caracterizar o sono, avaliar a variabilidade da frequência cardíaca e estresse oxidativo de cães braquicefálicos e mesocefálicos em ambiente domiciliar e hospitalar.

2 CAPITULO 1 – HEART RATE VARIABILITY AND SLEEP PATTERNS IN BRACHYCEPHALIC AND MESOCEPHALIC DOGS IN HOME AND HOSPITAL ENVIRONMENTS

2.1 ABSTRACT

The hospital environment can disrupt sleep-regulating mechanisms and alter physiological parameters, potentially reducing heart rate variability (HRV). The aim of the study was to assess 24-hour HRV, characterize, and compare sleep patterns in brachycephalic and mesocephalic dogs in both home and hospital settings. Six brachycephalic and six mesocephalic dogs were randomly selected for the study. Dogs underwent 24-hour ambulatory electrocardiography (Holter) and video recording overnight to characterize sleep periods. HRV in the time domain was evaluated over 24 hours and hourly. Nighttime behavior was categorized as sleeping, alert, active and barking. Brachycephalic dogs slept for 119 minutes, and mesocephalic dogs for 79 minutes. No difference in sleep time between different breed conformations was observed. However, brachycephalic dogs remained alert for a shorter time, 526 minutes, compared to mesocephalic dogs, 662 minutes, especially in the hospital environment. HRV did not change in the hospital setting, but dogs exhibited higher average heart rates and more episodes of tachycardia compared to the home environment. Mesocephalic dogs showed higher values of SDNN, SDANN, and SDNNI than brachycephalic dogs. NNs and NNNs had higher values in the late afternoon compared to the early morning hours, while average NN, SDNN, SDNNI, RMSSD, and pNN50 were higher during the early morning hours. Dog behavior in the hospital varied individually but established variables causing hospital stress resulted in physiological parameter changes and disrupted sleep mechanisms. In conclusion, brachycephalic and mesocephalic dogs exhibit similar sleep durations, the hospital environment does not affect sleep duration despite dogs spending more time alert. HRV remains unaffected in the hospital and is higher in mesocephalic dogs compared to brachycephalic dogs.

Keywords: Autonomic Balance; Circadian Rhythm; Holter

2.2 INTRODCUTION

A shared evolutionary history and socio-environmental involvement of dogs and humans have made the species a potential model for investigating various socio-cognitive activities of humans. Due to the established physiological parallels between humans and dogs,

studying these animals helps in understanding human neuropsychiatric conditions, including sleep, sleep disorders, and sleep-related respiratory disorders (BÓDIZS et al., 2020; NICOLAU et al., 2000; OVERALL; DUNHAM, 2013).

Is observed in mammals the presence of a master oscillator clock that hierarchically organizes a circadian timing system. In addition, peripheral oscillators can be found in various body tissues, giving rise to a system that evolves from molecular mechanisms within cells to organism-level structures (CALIANDRO et al., 2021). These mechanisms control biological rhythms, cyclic and relatively stable expressions that originate endogenously and can be influenced by environmental cycles. Through neural pathways, intrinsic biological clocks regulate sleep and wakefulness and control various homeostatic functions involving the sleep/wake cycle. These circadian clocks operate on approximately 24-hour cycles and are typically modulated by physiological oscillations (BARANWAL; YU; SIEGEL, 2023).

In dogs, the environment affects sleep time, and their nighttime behavior varies depending on their surroundings. Dogs living indoors spend 80% of their time sleeping, whereas dogs living outside without fencing spend only 60% of their time sleeping (ADAMS; JOHNSON, 1993). Ambient factors can indeed contribute to sleep disturbances. In humans, hospitalization often leads to sleep deficiency, characterized by poor sleep quality and restricted sleep time. Patients frequently report difficulties falling asleep, staying asleep, and overall dissatisfaction with their sleep quality due to extended periods of wakefulness at night (MANSOUR; KNAUERT, 2022).

The dogs are classified into brachycephalic, dolichocephalic, and mesocephalic based on their cranial measurements. Brachycephalic dogs are characterized by cranial alterations that result in a wide skull (MEOLA, 2013). Together with cranial alterations, these dogs are characterized by various anatomical changes such as stenosed nares, elongated soft palate, macroglossia, and hypoplastic trachea (DUPRÉ; HEIDENREICH, 2016; JONES; STANLEY; NELSON, 2020; KRAINER; DUPRÉ, 2022; SIEDENBURG; DUPRÉ, 2021). Such anatomical changes increase the susceptibility of these dogs to develop sleep disorders and sleep-related breathing disorders, resulting in chronic alterations in blood pressure, heart rate, risk of arrhythmias, cardiovascular diseases, and sudden death (DUCE et al., 2023; HENDRICKS et al., 1987a; NOCERA; CONNOLLY, 2023; PATEL et al., 2023).

Heart rate variability measurement is a non-invasive method for assessing the autonomic nervous system, through instantaneous fluctuations in heart rate (BIDOLI; ERHARD; DÖRING, 2022). Heart rate variability arises through a balance of activity in branches of the autonomic nervous system, which are influenced by neural, humoral, and physiological

mechanisms (VON BORELL et al., 2007). The autonomic balance can be assessed by different techniques. Temporal indices are easier to interpret and apply, as they quantify fluctuations over time in the intervals between cardiac cycles and can be used as a prognostic marker for cardiovascular risk (QIN et al., 2021; ZUPAN et al., 2016). The autonomic balance is influenced by circadian rhythms, with parasympathetic predominance during sleep facilitating, under normal conditions, a period of physiological recovery. Disruptions and disturbances during this period lead to sympathetic activation, preventing the complete renewal of these mechanisms (URBANIK et al., 2019).

Based on the above, we hypothesized that different environments may result in different sleep durations and changes in heart rate variability, and that brachycephalic breeds naturally have less sleep duration due to their anatomical alterations. The objective of this study was to evaluate heart rate variability in the time domain over 24 hours, characterize and compare sleep patterns in brachycephalic and mesocephalic dogs in both home and hospital environments.

2.3 MATERIALS AND METHODS

This study was conducted from December 2023 to May 2024. The research was carried out at the Superintendence University Veterinary Hospital Unit (SUHVU) of the Federal University of the Fronteira Sul - Campus Realeza, approved by the Ethics Committee on the Use of Animals (CEUA) of the university in question, under protocol number 8451180923. All the guardians responsible for the dogs signed a consent form about the research

2.3.1 Animals

Twelve dogs were used for the study, based on the sample calculation with a power of 0.82 (effect 0.8, α 0.05 and statistical power 80%) obtained using the G*Power software in version 3.1.9.7. The dogs were selected randomly depending on whether their owners joined the study. The screening process involved a physical examination, measurement of systolic blood pressure, echocardiogram and electrocardiogram. The inclusion criteria specified that the dogs had to be between one and six years old, healthy, brachycephalic or mesocephalic and without alterations in the screening process. Exclusion criteria included any type of illness, electrocardiographic or echocardiographic alterations, aggressive behavior and alterations in the screening process. The brachycephalic dogs selected comprised three Pugs, three Shih Tzu and the mesocephalic dogs comprised six mongrel dogs. They were on average 4.5 ± 1.8 years old and had a body condition score of 5 ± 1.

2.3.2 Experimental design

The dogs were divided into two groups of six each: brachycephalic dogs and mesocephalic dogs. The study consisted of 24-hour ambulatory electrocardiography (Holter) and recording of nocturnal behavior in home and hospital environments. Each dog was involved in the study for three days within a week. The environment in which the animal would begin the study was selected at random. On the first day, the dog underwent a Holter test and video recording during the night in one of the environments, followed by 24 hours of rest, and the study was resumed with the same tests in the opposite environment. The Holter test began at eight o'clock in the morning, the video recording at six o'clock in the afternoon and both ended at eight o'clock in the morning of the following day.

2.3.3 Environments

During the period of the experiment in the home environment, the dogs went about their normal routine. The dogs spent the night in their standard resting place, where the camera was positioned. Food was available according to the owners' choice and water was made available *ad libitum*. During the 24 hours in the hospital environment, the dog was housed in a stainless steel cage (120x80x60cm) in the SUHVU hospitalization room. The size of the room was 33m², the temperature was controlled by air conditioning and kept at 18ºC. During this period, the dog was fed with food provided by the guardians, using the animal's own containers when possible, and water was available *ad libitum*.

2.3.4 24-Hour Electrocardiography (Holter)

For the 24-hour electrocardiography test, a CardioLight® digital recorder from the brand Cardios® was used, obtained using a four-electrode, three-channel system. Before starting the test, the digital recorder was set up with the animal's data and the dogs were prepared by trichotomy and cleaning of the place where the electrodes were positioned, in the following order: the white electrode was positioned on the manubrium, the red electrode on the xiphoid cartilage, the black electrode on the left side of the thorax between the fifth and sixth intercostal space at the level of the costochondral junction, and the green electrode on the right side of the thorax in the same position as the black electrode. The electrodes were attached using bandages

and adhesive plaster and the digital recorder (62 grams) was positioned next to the animals' thoracic spine using protective clothing.

The test was analyzed semi-automatically using the CardioSmart® Professional CS 540 software, considering a maximum of 3% artifact in the test. The parameters assessed were heart rhythm, minimum, average and maximum heart rates, episodes of bradycardia (below 60 bpm) and tachycardia (above 170 bpm) and episodes of pauses (greater than one second) over 24 hours. In addition, heart rate variability (HRV) was analyzed by time domain over 24 hours and hourly. Table 1 summarizes the HRV indices assessed by the 24-hour Holter monitor.

Table 1. Description of the variables measured in 24-hour Holter heart rate variability in the time domain.

Index	Definition
NNs	Total number of cycles measured
NNNs	Total number of three successive normal cycles
Mean NN	Mean of all NN intervals
SDNN	Standard deviation of all NN intervals
SDANN	Standard deviation of mean NN intervals, measured in five-minute segments
SDNNI	Mean of the standard deviations of the NN intervals, measured in five-minute segments
RMSSD	Square root of the average of successive squared differences between adjacent NN
pNN50	Percentage of successive differences between NN intervals that are greater than 50 ms
TINN	Baseline width of the NN interval histogram

2.3.5 Characterization of Sleep

The sleep period and other behavioral categories were recorded by audiovisual recording with a TP-Link Tapo C200 digital surveillance camera. The camera was positioned directly facing the animal's standard resting place at home, and the inpatient cage in the hospital environment. The behavior recorded in the recordings was classified using the methodology of Adams & Johnson (1993) into five categories: Absent, Sleeping, Alert, Active and Barking (Figure 2). Table 2 summarizes the description of each behavioral category assessed during 18:00 and 08:00. The recordings were plotted in order to quantify the time the dogs spent in each category every second during the study period.

Table 2. Behavioral categories assessed during the 14-hour video recording.

Category	Description
Absent	When the dog leaves the camera frame, as no data can be obtained during these moments, these activities will be excluded from the calculations
Sleeping	Quiet sleep, when the dog is lying with its head on/between the thoracic limbs, in a lateral or dorsal decubitus position, with the neck muscles relaxed and completely still with its eyes closed Active sleep, when the dog is lying with its head down and neck muscles relaxed, but showing REM or spasmodic movements of the limbs, paws, ears, tail, tongue or muzzle. Vocalization by crying, yelping and muffled barking can accompany these patterns
Alert	When the dog has its eyes open and its neck muscles tense while lying down (in some cases with its ears up and searching for smells in the air), or sitting, in station, scratching, biting or licking itself, but remaining in its resting place
Active	When the dog is eating, drinking, defecating, urinating, playing, biting, digging, walking or running
Barking	When the awake dog emits a loud vocalization

(ADAMS; JOHNSON, 1994)

Figure 2 - Behaviors assessed in the video recording, during 18:00 and 08:00, in the home and hospital environment.

1= Absent; 2= Sleeping; 3= Alert; 4= Active; 5= Barking.

2.3.6 Statistical analysis

The data was analyzed using jamovi® software (version 2.5.5). The Shapiro-Wilk test was used to identify the distribution of variables. Parametric variables were presented as mean and standard deviation, non-parametric variables as median and interquartile ranges (25-75%). A Generalized Linear Model (GLM) was conducted using Omnibus ANOVA, followed by Bonferroni post hoc, which assessed differences and interactions between the independent variables, brachycephalic and mesocephalic group, home and hospital environments, and time of day, for the dependent variables Absent, Sleeping, Alert, Active and Barking, and HRV

parameters in the time domain as: NNs, mean NN, SDNN, SDANN, SDNNI, NNNs, RMSSD, TINN, pNN>50%, HR Min, HR Med, HR Max, Bradycardia Episodes, Tachycardia and Pauses. The significance level considered was $p<0.05$.

2.4 RESULTS

The predominant rhythm found was sinus arrhythmia/sinus rhythm, with no atrioventricular blocks, atrial complexes or premature ventricular complexes.

2.4.1 Comparison between brachycephalic and mesocephalic dogs, and home and hospital environments

Table 3. Proportions of time recorded for the behaviors evaluated at night, between 18:00 and 08:00, of brachycephalic and mesocephalic dogs, and home and hospital environments.

	Groups			Environments		
	Brachycephalic	**Mesocephalic**	**p**	**Home**	**Hospital**	**p**
Absent	2.5%	4.6%	0.350	24.9%†	0%†	0.001
Sleeping	17.4%	10%	0.741	11.4%	8.5%	0.960
Alert	76.3%*	83.6%*	0.019	61.9%†	88.6%†	0.003
Active	3.8%	1.8%	0.651	1.8%	2.9%	0.923
Barking	0%	0%	0.367	0%	0%	0.071
Total	**100%**	**100%**		**100%**	**100%**	

* Difference between brachycephalic and mesocephalic dogs; † Difference between hospital and home environments.

The brachycephalic dogs remained asleep for 17.4% of the time recorded and most of the time the dogs remained alert (76.3%). Mesocephalic dogs, on the other hand, spent 10% of the time recorded asleep and 83.6% alert. Table 3 shows the percentages of time recorded for the behaviors assessed at night, during 14 hours, of brachycephalic and mesocephalic dogs, and home and hospital environments. The sleep time of brachycephalic dogs was similar to that of mesocephalic dogs ($p=0.741$), however, brachycephalic dogs spent less time alert compared to mesocephalic dogs ($p=0.019$). In terms of active and barking time, brachycephalic and mesocephalic dogs had similar times ($p=0.651$) ($p=0.367$).

When evaluating the effect of environment, the dogs slept similarly in the home and hospital environments ($p=0.960$), sleeping 11.4% of the time recorded in the home and 8.5% in the hospital. However, they were more alert in the hospital environment compared to the home environment ($p=0.003$), remaining alert 88.4% of the time recorded in the hospital and 61.9% at home. There were no difference between times for active behavior ($p=0.923$) and barking

(p=0.071) in both environments. Figures 3 and 4 summarize the time duration of the behaviors of brachycephalic and mesocephalic dogs assessed in the home and hospital environments, between 18:00 and 08:00. Table 4 shows the behaviors of brachycephalic and mesocephalic dogs evaluated during the night, between 18:00 and 08:00, in the home and hospital environments.

Table 4 - Means ± SD and medians (25-75%) in minutes of the behaviors evaluated during the night, between 18:00 and 08:00, of brachycephalic and mesocephalic dogs, and home and hospital environments.

	Groups			Environments		
	Brachycephalic	**Mesocephalic**	**p**	**Home**	**Hospital**	**p**
Absent	17.1 (0.0-192.3)	35.9 (0.0-112.7)	0.350	203.4 ± 153.6†	0.0 (0.0-0.5) †	0.001
Sleeping	119.7 ± 108	79.8 (49.8-104.5)	0.741	93.4 (53.5-119.8)	66.4 (8.5-140.5)	0.960
Alert	526.1 ± 155.6*	662.0 ± 157.7*	0.019	503.7 ± 117.1†	684.4 ± 166.1†	0.003
Active	25.7 ± 23.4	13.6 (4.2-27.6)	0.651	13.6 (6.7-34.9)	19.1 (6.1-32.6)	0.923
Barking	0.0 (0.0-0.0)	0.0 (0.0-0.7)	0.367	0.0 (0.0-0.0)	0.0 (0.0-36.6)	0.071

* Difference between brachycephalic and mesocephalic dogs; † Difference between hospital and home environments.

Figure 3 – Behaviors of dogs evaluated at night, between 18:00 and 08:00, comparing brachycephalic and mesocephalic dogs

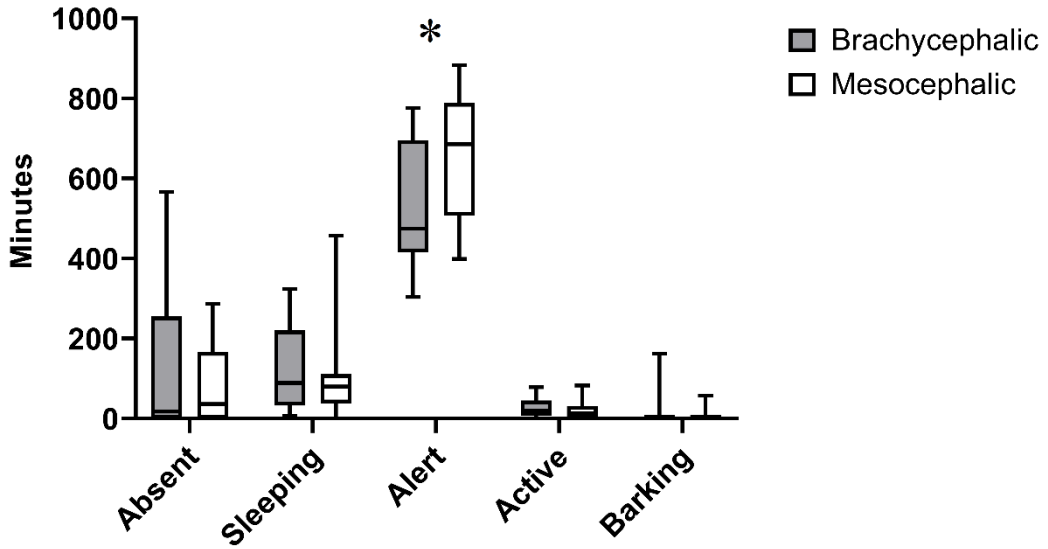


Figure 4 - Behaviors of dogs evaluated at night, between 18:00 and 08:00, comparing home and hospital environments

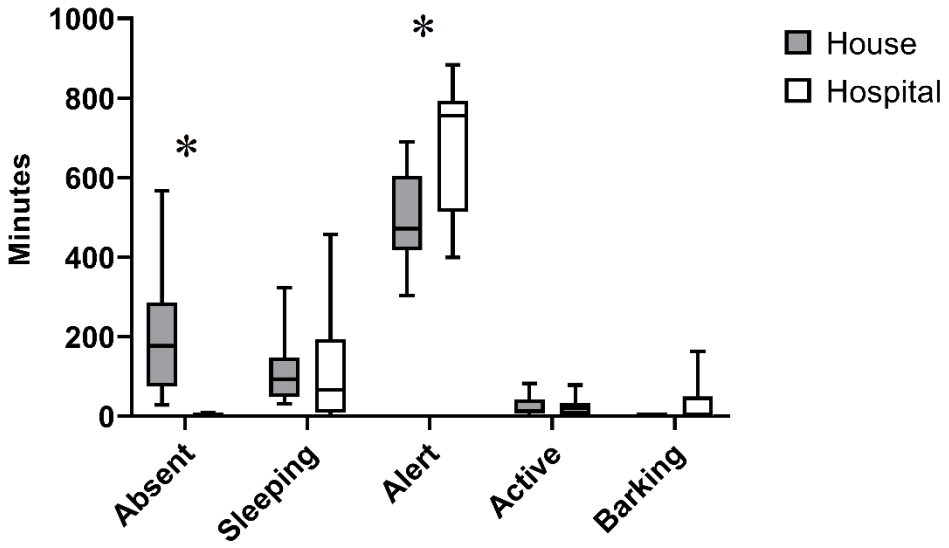


Table 5 shows the heart rate variability values in the time domain assessed over 24 hours, comparing brachycephalic and mesocephalic dogs, and hospital and home environments. The group of brachycephalic dogs had lower SDNN (p=0.011), SDANN (p=0.019) and SDNNI (p=0.027) values when compared to mesocephalic dogs. They also had higher minimum heart rate values (p=0.019). The dogs that spent the day in the hospital environment had higher NNs (p=0.023), mean heart rate (p=0.012) and tachycardia episodes (p=0.006) when compared to the home environment. In addition, they had lower mean NN values (p=0.041) and pNN50 (p=0.020) in the hospital environment when compared to the home environment.

There were no differences between groups or environments for the duration of NNNs, RMSSD, TINN, Maximum Heart Rate, Bradycardia episodes and Pauses. There were no differences between groups for NNs, mean NN, pNN50, mean heart rate and tachycardia episodes. There were no differences between environments for SDNN, SDANN, SDNNI and Minimum Heart Rate. There were no interactions between environments and groups for any of the variables (p>0.05).

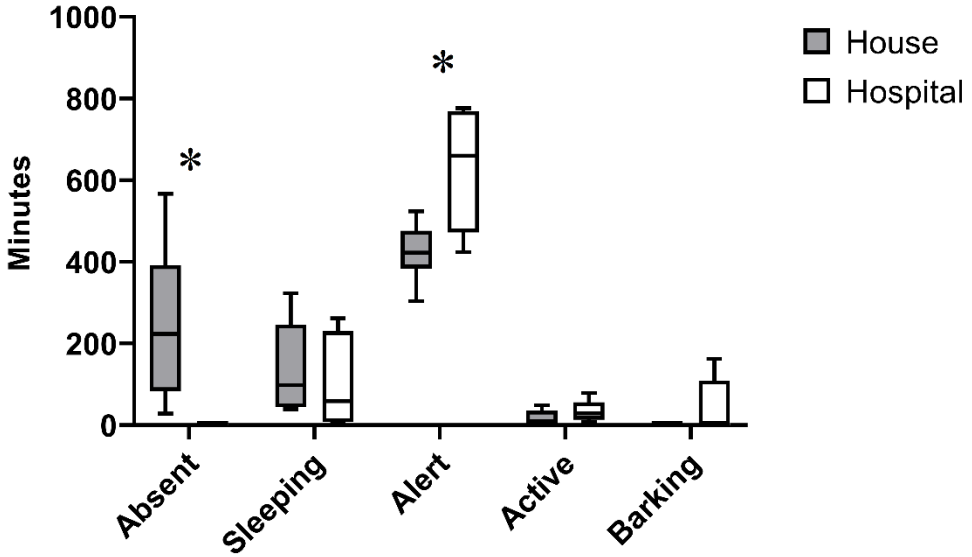
Table 5 - Heart rate variability indices in the time domain, minimum, mean and maximum heart rates, bradycardia and tachycardia episodes, and pauses recorded on a 24-hour Holter compared between brachycephalic and mesocephalic dogs, and hospital and home environments.

	Groups		Environments	
	Brachycephalic	**Mesocephalic**	**Hospital**	**Home**
NNs (nº)	103919 ± 26673	88711 ± 31599	109839 ± 24039†	82791 ± 29312†
NNNs (nº)	93924 ± 34981	91491 (45478-110376)	111927 (89375-122143)	84465 (45478-100155)
Mean NN (ms)	737 ± 152	807 ± 208	626 (587-732)†	849 ± 157†
SDNN (ms)	229 ± 75*	313 ± 73*	255 ± 90	287 ± 78
SDANN (ms)	159 ± 40*	206 ± 52*	186 ± 64	179 ± 37
SDNNI (ms)	168 ± 74*	237 ± 72*	175 (134-199)	226 ± 72
RMSSD (ms)	124 ± 47	167 ± 63	157 ± 50	135 ± 67
TINN (ms)	613 (520-677)	886 ± 523	613 (540-804)	613 (420-1343)
pNN50 (%)	53 ± 14	58 ± 17	48 ± 13†	63 ± 14†
HR Min (bpm)	40 (39-48)*	37 ± 4*	42 ± 7	39 ± 5
Average HR (bpm)	88 ± 15	82 ± 19	94 ± 16†	76 ± 13†
Max HR (bpm)	250 (250-250)	248 (220-250)	250 (230-250)	250 (242-250)
Bradycardia	121 ± 92	132 (104-138)	96 ± 63	160 ± 85
Tachycardia	108 ± 77	37 (22-175)	179 ± 139†	49 ± 33†
Pauses	18232 ± 13619	24775 ± 9338	17342 ± 10857	25665 ± 11847

* Difference between brachycephalic and mesocephalic dogs; † Difference between hospital and home environments.

2.4.2 Effect of the environment on brachycephalic and mesocephalic dogs

Figure 5 - Behaviors of brachycephalic dogs evaluated at night, between 18:00 and 08:00, comparing home and hospital environments

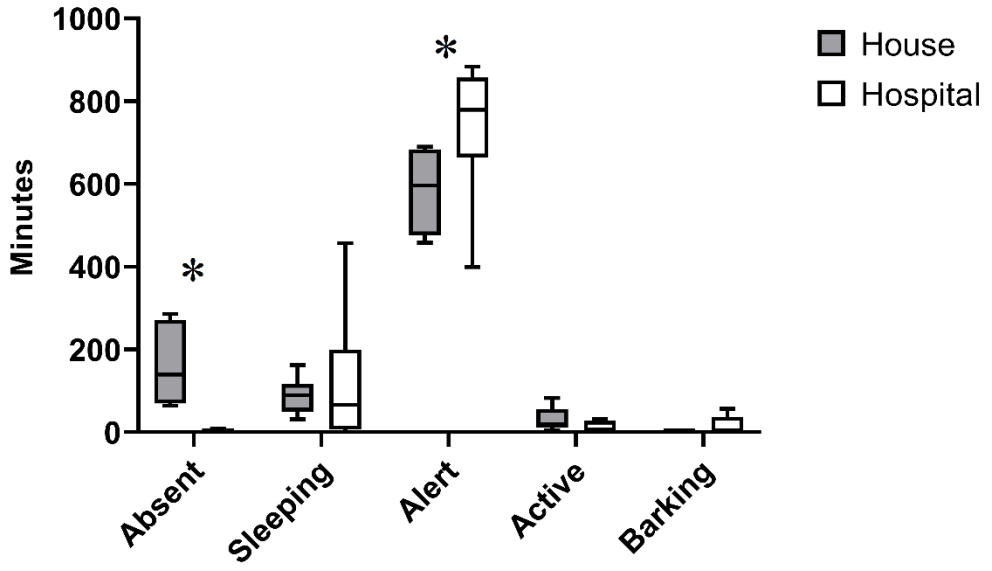


The brachycephalic dogs had similar sleeping times when they spent the night in hospital and at home (p=0.597), however, they spent more time alert in the hospital environment than at home (p=0.013). They also spent similar amounts of time active (p=0.216) and barking (p=0.165) in both environments. The dogs spent more time absent in the home environment than in the hospital environment (p=0.011) (Figure 5). NNs, mean NN, SDNN, SDANN, SDNNI, NNNs, RMSSD, TINN and pNN50, HR Min, HR Med, HR Max, bradycardia episodes, tachycardia episodes and pause episode showed no differences between environments. Table 6 shows the values for heart rate variability in the time domain assessed over 24 hours in brachycephalic dogs comparing hospital and home environments.

Table 6: Heart rate variability indices in the time domain, minimum, mean and maximum heart rates, bradycardia and tachycardia episodes, and pauses recorded on the 24-hour Holter of brachycephalic dogs, between hospital and home environments.

	Hospital	**Home**
NNs (nº)	117938 ± 17744	89900 ± 27905
NNNs (nº)	103410 ± 38220	84437 ± 31864
NN médio (ms)	626 (597-629)	816 ± 148
SDNN (ms)	195 ± 37	262 ± 91
SDANN (ms)	147 ± 36	170 ± 43
SDNNI (ms)	133 ± 40	202 ± 87
RMSSD (ms)	128 ± 33	120 ± 61
TINN (ms)	612 ± 130	684 ± 423
pNN50 (%)	45 ± 9	60 ± 14
HR Min (bpm)	46 ± 7	41 ± 5
Average HR (bpm)	96 ± 14	81 ± 12
Max HR (bpm)	250 (250-250)	250 (250-250)
Bradycardia	86 ± 85	156 ± 91
Tachycardia	148 ± 92	69 ± 26
Pauses	12392 ± 11230	24071 ± 14145

Figure 6 - Behaviors of mesocephalic dogs evaluated at night, between 18:00 and 08:00, comparing home and hospital environments.



The mesocephalic dogs showed similar times of sleep (p=0.682), alertness (p=0.085), activity (p=0.178) and barking (p=0.162) when they spent the night in hospital and at home. The dogs spent more time absent in the home environment than in the hospital environment (p=0.003) (Figure 6). The mesocephalic dogs had more episodes of tachycardia in the hospital environment (210 ± 178) than in the home environment (30 ± 29) (p=0.036). NNs, mean NN, SDNN, SDANN, SDNNIX, NNNs, RMSSD, TINN and pNN>50%, HR Min, HR Med, HR Max, bradycardia episodes and pause episode did not differ between environments. Table 7 shows the values for heart rate variability in the time domain assessed over 24 hours in mesocephalic dogs comparing hospital and home environments.

Table 7. Heart rate variability indices in the time domain, minimum, mean and maximum heart rates, bradycardia and tachycardia episodes, and pauses recorded on the 24-hour Holter of mesocephalic dogs, between hospital and home environments.

	Hospital	Home
NNs (nº)	101740 ± 28268	75682 ± 31469
NNNs (nº)	96320 ± 29979	69755 ± 34081
NN médio (ms)	662 (589-751)	882 ± 172
SDNN (ms)	286 (263-314)	312 ± 61
SDANN (ms)	225 ± 65	188 ± 31
SDNNI (ms)	199 (190-213)	250 ± 49
RMSSD (ms)	185 ± 50	150 ± 75
TINN (ms)	821 ± 542	951 ± 547
pNN50 (%)	51 ± 16	66 ± 15
HR Min (bpm)	38 ± 5	37 ± 3
Average HR (bpm)	92 ± 20	72 ± 14
Max HR (bpm)	233 ± 21	248 (229-250)
Bradycardia	106 ± 34	163 ± 87
Tachycardia	210 ± 178*	30 ± 29*
Pauses	22292 ± 8626	27258 ± 10130

*Differences between environments

2.4.3 Hour by hour difference

Table 8 summarizes the duration of time in minutes for the behaviors assessed during the period from 18:00 to 08:00, every hour. Table 9 summarizes the heart rate variability indices in the time domain recorded on the 24-hour Holter, every hour. Sleep time recorded at 24:00 was longer than at 19:00 ($p=0.011$). The time spent alert, active and barking remained constant between the hours of recording ($p>0.05$). The absent time recorded at 18:00 was greater than 01:00, 02:00, 03:00, 04:00, 05:00, 06:00, 07:00, 08:00, 22:00, 23:00 and 24:00 ($p<0.05$), the 19:00 record was greater than 02:00, 03:00, 04:00, 05:00, 07:00, 23:00 and 24:00 ($p<0.05$). There were no interactions between time of day, groups and environments ($p>0.05$).

In relation to the heart rate variability variables in the time domain, the NNs varied in relation to the time of day, the values recorded at 17:00 and 18:00 were higher than 03:00, 04:00, 05:00 and 07:00 ($p<0.05$) and the value recorded at 07:00 was lower than 09:00, 10:00, 16:00 and 19:00 ($p<0.05$). The average NN value recorded at 03:00 was higher than 08:00 and 09:00 ($p<0.05$), the record at 04:00 was higher than 16:00, 17:00, 18:00 and 19:00 ($p<0.05$) and at 05:00 higher than 08:00, 09:00, 17:00, 18:00 and 19:00 ($p<0.05$). The NNNs recorded at 03:00, 04:00 and 05:00 were lower than 17:00 and 18:00 ($p<0.05$) and the value recorded at 07:00 was lower than 09:00, 17:00, 18:00 and 19:00 ($p<0.05$).

The SDNN value at 03:00 was higher than 08:00, 09:00, 10:00, 12:00, 13:00, 14:00, 16:00, 17:00 and 18:00 ($p<0.05$), at 04:00 higher than 08:00, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:00, 18:00 and 19:00 ($p<0. 05$), at 05:00 higher than 08:00, 09:00, 10:00,

12:00, 13:00, 14:00, 16:00, 17:00, 18:00 and 19:00 ($p<0.05$) and the value recorded at 06:00 was higher than 08:00, 09:00, 17:00 and 18:00 ($p<0.05$)

The SDANN index did not vary between times of day ($p=0.171$). The SDNNI value recorded at 02:00 was higher than 17:00 and 18:00 ($p<0.05$), at 03:00 higher than 08:00, 09:00, 12:00, 13:00, 16:00, 17:00 and 18:00 ($p<0. 05$), at 04:00 higher than 08:00, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:00, 18:00, 19:00 and 20:00 ($p<0. 05$), at 05:00 higher than 08:00, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:00, 18:00 and 19:00 ($p<0.05$) and at 06:00 higher than 08:00, 09:00, 17:00 and 18:00 ($p<0.05$).

The RMSSD values varied, the records at 01:00 and 02:00 were higher than 08:00, 09:00, 10:00, 16:00, 17:00 and 18:00 ($p<0.05$). The values recorded at 03:00, 04:00 and 05:00 were higher than 08:00, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:00, 18:00 and 19:00 ($p<0.05$). The TINN value did not vary during the hours of the day ($p=0.223$). The pNN50 value recorded at 01:00 was higher than 17:00 and 18:00 ($p<0. 05$), at 02:00 and 03:00 was higher than 08:00, 09:00, 16:00, 17:00 and 18:00 ($p<0.05$) and the value recorded at 04:00 and 05:00 was higher than 08:00, 09:00, 12:00, 16:00, 17:00, 18:00 and 19:00 ($p<0.05$). There were no interactions between times of day, groups and environments ($p>0.05$).

Table 8. Medians (25-75%) in minutes, of the behaviors recorded from 18:00 to 08:00, every hour, of brachycephalic and mesocephalic dogs in the home and hospital environment.

Hour	Absent	Sleep	Alert	Active	Barking
18:00	35.1 (0.0-58.7)^{A}	0.0 (0.0-0.0)	14.7 (0.0-47.0)	0.2 (0.0-4.3)	0.0 (0.0-0.0)
19:00	0.0 (0.0-38.7)^{B}	0.0 (0.0-0.0)^{a}	55.3 (23.2-60.0)	0.09 (0.0-1.8)	0.0 (0.0-0.0)
20:00	0.0 (0.0-18.6)	0.0 (0.0-0.0)	47.4 (22.9-57.6)	0.3 (0.0-2.3)	0.0 (0.0-0.0)
21:00	0.0 (0.0-7.86)	0.0 (0.0-3.7)	38.3 (23.9-58.0)	0.09 (0.0-3.7)	0.0 (0.0-0.0)
22:00	0.0 (0.0-0.0)^{a}	2.8 (0.0-11.1)	51.5 (38.4-57.3)	0.0 (0.0-1.8)	0.0 (0.0-0.0)
23:00	0.0 (0.0-0.0)^{ab}	10.6 (0.0-17.7)	45.3 (36.5-56.5)	0.0 (0.0-1.9)	0.0 (0.0-0.0)
24:00	0.0 (0.0-1.1)^{ab}	10.6 (1.8-19.5)^{A}	46.0 (25.4-51.5)	0.1 (0.0-1.7)	0.0 (0.0-0.0)
01:00	0.0 (0.0-0.0)^{a}	4.3 (0.0-16.6)	43.3 (27.4-58.5)	0.1 (0.0-1.4)	0.0 (0.0-0.0)
02:00	0.0 (0.0-0.0)^{ab}	3.2 (0.0-28.5)	50.5 (26.6-56.8)	0.0 (0.0-0.2)	0.0 (0.0-0.0)
03:00	0.0 (0.0-0.0)^{ab}	6.9 (0.0-15.6)	49.5 (43.4-59.4)	0.0 (0.0-0.4)	0.0 (0.0-0.0)
04:00	0.0 (0.0-0.0)^{ab}	5.2 (0.0-22.8)	46.0 (37.1-59.4)	0.0 (0.0-0.02)	0.0 (0.0-0.0)
05:00	0.0 (0.0-0.0)^{ab}	0.0 (0.0-8.5)	51.4 (41.1-60.0)	0.0 (0.0-0.5)	0.0 (0.0-0.0)
06:00	0.0 (0.0-0.0)^{a}	0.0 (0.0-19.4)	52.1 (29.0-57.5)	0.0 (0.0-1.4)	0.0 (0.0-0.0)
07:00	0.0 (0.0-0.0)^{ab}	0.0 (0.0-0.9)	56.8 (49.6-59.9)	0.1 (0.0-1.9)	0.0 (0.0-0.0)
08:00	0.0 (0.0-0.0)^{a}	0.0 (0.0-0.0)	60.0 (54.3-60.0)	0.0 (0.0-0.5)	0.0 (0.0-0.0)

The presence of equal letters in different types of case (upper and lower case) within the columns indicates a statistical difference ($p<0.05$).

Table 9. Means (±SD) and medians (25-75%) of heart rate variability indexes in the time domain recorded over 24 hours, evaluated hourly, of brachycephalic and mesocephalic dogs in the home and hospital environment.

Hour	NNs	NNNs	NN médio	SDNN	SDANN	SDNNI	RMSSD	TINN	pNN>50%
01:00	3487 ± 1333	3215 ± 1474	894 ± 200	272 ± 105	75 (50-105)	242 ± 99	315 ± 141^{A}	437 (302-952)	76 (60-80)^{A}
02:00	3312 ± 1312	3032 ± 1479	920 ± 214	276 ± 104	75 (53-106)	252 ± 102^{A}	315 ± 137^{B}	562 (369-1060)	74 (67-84)^{B}
03:00	3046 (1990-3789)^{ab}	2685 (1558-3699)^{A}	974 ± 208^{A}	306 ± 109^{A}	100 ± 53	268 ± 111^{B}	341 ± 145^{C}	814 (326-1301)	73 ± 11^{C}
04:00	3001 ± 1125^{ab}	2717 ± 1257^{B}	985 ± 221^{B}	320 ± 115^{B}	107 (90-163)	288 ± 117^{C}	344 ± 151^{D}	760 (442-1352)	74 ± 10^{D}
05:00	3101 ± 1239^{ab}	2813 ± 1372^{C}	891 (802-1187)	311 ± 108^{C}	115 ± 57	280 ± 111^{D}	339 ± 142^{E}	680 (453-1368)	74 ± 12^{E}
06:00	3654 ± 1632	3385 ± 1727	888 ± 293	293 ± 120^{D}	132 ± 70	256 ± 120^{E}	295 ± 151	542 (384-981)	66 ± 18
07:00	2828 ± 1621^{abC}	2655 ± 1636^{D}	814 ± 297	254 ± 124	110 ± 73	225 ± 118	255 ± 161	594 (480-927)	59 ± 23
08:00	3320 ± 1904	3181 ± 1952	709 ± 229^{a}	159 (106-221)^{abcd}	74 ± 45	141 (86-193)^{bcde}	142 (90-240)^{abcde}	453 (356-657)	51 ± 24^{bcde}
09:00	4714 ± 1870^{c}	4540 ± 1966^{d}	713 ± 210^{a}	155 (110-236)^{abcd}	86 ± 51	130 (92-196)^{bcde}	136 (94-219)^{abcde}	504 (364-747)	50 ± 22^{bcde}
10:00	4554 ± 1688^{c}	4396 ± 1786	766 ± 213	184 (121-246)^{abc}	81 (54-111)	164 (104-218)^{cd}	190 ± 96^{abcde}	545 (406-717)	55 ± 19
11:00	4381 ± 1689	4121 ± 1912	766 ± 221	210 ± 92^{b}	86 (62-133)	155 (110-212)^{cd}	202 ± 116^{cde}	576 (327-978)	56 ± 20
12:00	4441 ± 2027	4217 ± 2171	767 ± 261	194 ± 104^{abc}	82 ± 43	170 ± 98^{bcd}	198 ± 128^{cde}	541 (325-1021)	53 ± 25de
13:00	4471 ± 1928	4278 ± 2035	771 ± 239	197 ± 89^{abc}	77 (54-103)	170 ± 85^{bcd}	199 ± 110^{cde}	558 (395-809)	55 ± 22
14:00	4374 ± 1834	4165 ± 1953	779 ± 263	198 ± 85^{abc}	87 ± 36	173 ± 82^{cd}	201 ± 114^{cde}	709 ± 409	56 ± 25
15:00	4397 ± 1837	4192 ± 1931	717 (560-1035)	210 ± 90^{b}	97 (66-152)	179 ± 91^{cd}	207 ± 125^{cde}	541 (398-973)	56 ± 26
16:00	4577 ± 1932^{c}	4397 ± 2041	658 (569-889)b	180 (128-233)^{abc}	64 (49-96)	166 ± 88^{bcd}	185 ± 103^{abcde}	504 (355-616)	50 ± 23^{bcde}
17:00	4818 ± 1874^{A}	4652 ± 1960^{abcd}	623 (542-764)^{b}	182 ± 85^{abcd}	68 (48-121)	151 ± 75^{abcde}	168 ± 103^{abcde}	471 (318-658)	46 ± 23^{abcde}
18:00	4842 ± 1669^{B}	4698 ± 1723^{abcd}	632 (563-800)^{b}	175 (115-220)^{abcd}	76 (49-122)	127 (99-196)^{abcde}	173 ± 101^{abcde}	459 (370-618)	47 ± 21^{abcde}
19:00	4610 ± 1449^{c}	4431 ± 1571^{d}	717 ± 152^{b}	207 ± 93^{bc}	85 (60-123)	171 ± 85^{cd}	202 ± 117^{cde}	513 (430-653)	53 ± 21de
20:00	4122 ± 1484	3864 ± 1616	754 ± 227	218 ± 98	70 (56-112)	187 ± 99^{c}	230 ± 124	625 (342-945)	57 ± 24
21:00	4105 ± 1587	3902 ± 1714	793 ± 203	222 ± 99	76 (49-110)	193 ± 94	236 ± 118	458 (327-881)	60 ± 19
22:00	3794 ± 1340	3567 ± 1484	836 ± 183	245 ± 100	83 (51-125)	218 ± 97	265 ± 126	535 (383-905)	66 ± 15
23:00	3581 ± 1504	3465 ± 1463	852 ± 185	249 ± 98	93 ± 53	224 ± 98	272 ± 114	530 (337-916)	67 ± 16
24:00	3778 ± 1387	3558 ± 1528	845 ± 194	243 ± 102	92 ±56	215 ± 99	271 ± 123	542 (364-1090)	66 ± 17

NNs and NNNs = whole number of cardiac cycles. Mean NN, SDNN, SDANN, SDNNI, RMSSD and TINN = milliseconds (ms). pNN50 = percentage (%). The presence of the same letters in different case types (upper and lower case) within the columns indicates a statistical difference ($p<0.05$).

2.5 DISCUSSION

A dog's sleep cycle is approximately 20 minutes long and occurs several times during the night, mainly between 21:00 and 06:00 hours (ADAMS & JOHNSON, 1993; BÓDIZS et al., 2020). The fact that dogs sleep predominantly during the night justifies the study to evaluate behaviors during the period from 18:00 to 08:00 in order to characterize the dogs' sleep during this period, however as the dogs' sleep cycle is only 20 minutes it was not possible to correlate heart rate variability with sleep time itself.

The sleep of mammals is extremely complex, and the sleep duration of different species varies greatly. Theories suggest that mammalian sleep can be calculated based on their metabolic processes associated with body size, estimating that domestic dogs sleep an average of 10 hours per day (BÓDIZS et al., 2020; SAVAGE; WEST, 2007). Such values can range from 7 to 16 hours (BÓDIZS et al., 2020; CAMPBELL; TOBLER, 1984). A study that evaluated dogs' sleep using an accelerometer showed that dogs spent an average of 6 hours at rest (PATEL et al., 2017). In our study, the dogs only slept for 93 (53-119) minutes in the home environment and 66 (8-140) minutes in the hospital environment, much less than expected. These dogs ended up spending more time in alert behavior during the night, spending 503 ± 117 minutes in the home environment and 684 ± 166 minutes in the hospital environment.

Based on our hypothesis and evidence from the literature, it was expected that dogs would sleep less in the hospital environment, but sleep time did not differ between home and hospital environments. Physiological parameters in dogs, such as mean arterial pressure, rectal temperature and pulse rate are altered in the hospital environment, mainly due to the stressful factors of the change of environment (BRAGG et al., 2015). In humans, there is already evidence of poor sleep quality in hospitalized individuals, with various factors causing disturbances, such as noise, uncomfortable beds, bright lights and interruptions from nurses (BEVAN et al., 2019; DOBING et al., 2016; LEI et al., 2009).

It was expected that brachycephalic dogs would have a reduced sleep time, with sleep interruptions, due to anatomical conditions that make it difficult for these breeds to breathe. Brachycephalic dogs of the English Bulldog breed, which are considered models for sleep apnea in humans, show constant sleep interruptions due to severe respiratory obstruction, which result in increased sleepiness throughout the day and an ease of sleeping in different environments

(HENDRICKS et al., 1987). However, in our study, sleep time did not differ between brachycephalic and mesocephalic dogs, and the environment had no effect on their sleep time.

The normal sleep schedule of dogs may justify the longer period of absence of the video recording observed at 18:00 and 19:00, since the recording was only carried out at the animal's sleeping place, and dogs have very similar sleep schedules to humans, normally occurring between 21:00 and 06:00 (BÓDIZS et al., 2020). Dogs have a more flexible schedule, which is due to weaker circadian regulation, allowing them to follow their owners' schedules and adjust to the routine (ADAMS; JOHNSON, 1994; HAWKING et al., 1971). However, despite the absence of the guardian in the hospital environment, the dogs showed no difference in sleep times between the home and hospital environments, since no interactions were found between the time of recording and the environment.

It is interesting to note that HRV indices are considered intrinsic and are strongly linked to the individual characteristics of each dog. In a study carried out by Doxey & Boswood (2004), brachycephalic dogs showed higher vasovagal tone index (VVTI) values when compared to non-brachycephalic dogs. In the study by Fernandes et al. (2024) this was not observed, where brachycephalic dogs did not have higher VVTI values than non-brachycephalic dogs. In our study, brachycephalic dogs had lower SDNN, SDANN and SDNNI values than mesocephalic dogs.

Brachycephalic dogs have several electrocardiographic alterations resulting from the predominance of the parasympathetic autonomic system, the presence of sinus arrhythmia with an R-R interval variation of more than 20%, the presence of pauses ranging from four to six seconds and migratory pacemaker are the main alterations, observed less frequently in mesocephalic dogs (DIAS et al., 2016). In our study, all the dogs had sinus arrhythmia, but brachycephalic dogs had a higher median minimum heart rate than mesocephalic dogs.

In a study carried out by Ferasin, Ferasin and Little (2010) which evaluated the heart rate of dogs in electrocardiographic recordings, a low correlation of heart rate with body condition score or weight was identified. In the same study, it was found that the average heart rate did not differ in relation to breed morphology, with brachycephalic, mesocephalic and dolichocephalic dogs having similar values. This evidence corroborates the findings of our study, in which brachycephalic dogs had similar mean heart rate values compared to mesocephalic dogs. However, in another study, brachycephalic dogs showed higher heart rate values than non-brachycephalic dogs, both before and after physical exercise (TARELHO et al., 2023).

In our study, when the dogs remained in the hospital environment during the day, there were higher NN values and lower mean NN values, suggesting a higher heart rate than in the home environment. The behavior of dogs in a hospital environment can vary and is usually individual. Some animals can be more active, showing changes such as excessive barking, attempts to escape, increased heart rate and lower HRV values when compared to more passive dogs, which do not show such changes (VÄISÄNEN et al., 2005). When it comes to physiological parameters, blood pressure, rectal temperature and pulse rate increase in a hospital environment when compared to a home environment (BRAGG et al., 2015).

Veterinary hospitals and kennels have sounds and lighting that are characteristic of the environment and which act as a stress factor for hospitalized dogs. Lighting becomes an important factor because it is from there that the circadian rhythm is controlled, the constant presence of which results in a misalignment of the circadian rhythm and possible changes in heart rate variability, temperature, vascular tone and immunity (LEFMAN; PRITTIE, 2019). In humans, HRV can be used as a predictor of mortality in ICU patients, making such indices extremely important in hospitalized dogs (BODENES et al., 2022). The dogs in our study showed a reduction in only one HRV index in the hospital environment, pNN50, which indicates a reduction in parasympathetic system activation.

In hospitalized dogs, psychogenic stress leads to an increase in heart rate; however, this increase is not a specific marker of stress. Meanwhile, HRV remains reduced during these stressful moments, a phenomenon observed in dogs, humans, and laboratory animals in hospital settings (HEKMAN; KARAS; SHARP, 2014). The stress of the hospital environment may explain the higher number of episodes of tachycardia observed in dogs in hospital settings.

The parameters of HRV undergo circadian variation, with higher values during the night and consequently lower values during the day (HASEGAWA et al., 2024; OLSEN et al., 1999). In our study, higher values of NN intervals were obtained at 17:00 and 18:00, indicating a greater number of cardiac cycles during that period and consequently a higher heart rate. In agreement with this, lower values of NNN intervals were obtained at 03:00, 04:00, 05:00, and 06:00, indicating a lower number of cardiac cycles during that period and consequently a lower heart rate.

In humans, it is established that different sleep stages exhibit varying predominance of the autonomic nervous system. Sleep itself can be influenced by the multiple bidirectional interactions of this system, resulting in different states of homeostasis during sleep. Despite shifts in autonomic dominance, several sleep stages show parasympathetic predominance, leading to lower heart rates and higher heart rate variability indices during the nighttime

(BONNET; ARAND, 1997; BUŠEK et al., 2005; TOBALDINI et al., 2013). In our study, higher mean NN, SDNN, SDNNI, RMSSD, and pNN50 values were found during the early morning hours, between 01:00 and 06:00.

Studies that evaluated heart rate over 24 hours and during sleep have shown results where heart rate during REM and non-REM sleep is reduced, with the lowest heart rate values occurring in the early morning between 02:00 and 05:00 (OLSSON et al., 2003; VARGA et al., 2018), however, the dogs in the study showed extremely limited sleep time. The reduction in HRV during the early morning period may be justified by the decrease in activities occurring in the hospital, with the dog remaining in a quieter environment compared to daytime.

Some limitations of the study should be addressed and taken into consideration. The characterization of sleep may have been affected by the presence of bandages, adhesive tapes, and the protective clothing of the Holter monitor; therefore, it would be necessary to record the dogs' sleep period without the Holter monitor in both environments. The lack of standardization of airway obstruction severity in brachycephalic dogs prevents obtaining a completely homogeneous group and comparable, precise results between study groups. Regarding HRV, the minimum duration of pause episodes, set to just one second, may have reduced the specificity of the variable and hindered a more accurate analysis of it.

2.6 CONCLUSION

The study demonstrated that breed conformation and environment do not affect nighttime sleep duration when comparing brachycephalic and mesocephalic dogs in both home and hospital environments. However, dogs spent more time in an alert state and exhibited higher average heart rates and more episodes of tachycardia in the hospital environment compared to the home environment. Additionally, the study showed the absence of vagal stimulation in brachycephalic dogs and that the hospital environment does not affect heart rate variability in brachycephalic and mesocephalic dogs.

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3 CAPITULO 2 – EFFECT OF ENVIRONMENT, BREED CONFORMATION, AND CIRCADIAN RHYTHM ON OXIDATIVE STRESS IN DOGS

3.1 ABSTRACT

The objective of the study was to evaluate oxidative stress in brachycephalic and mesocephalic dogs in both home and hospital environments. Five brachycephalic and seven mesocephalic dogs were randomly selected to participate in the study. Blood samples were collected from the dogs at night and in the morning in both home and hospital settings. Oxidative stress was assessed by measuring plasma levels of lipid peroxidation using the TBARS technique, as well as measuring vitamin C concentration and protein thiol levels. Levels of lipid peroxidation were higher in the hospital environment compared to the home environment, with malondialdehyde (MDA) concentration in the hospital environment measuring 8.08 (4.02-14.9) nmol/mL, compared to 6.04 (3.78-8.30) nmol/mL in the home environment (p=0.046). When evaluating the effect of environment and sampling time in brachycephalic dogs, higher MDA concentrations were observed in the hospital environment (9.36 ± 4.24 nmol/mL) compared to the home environment (4.55 ± 1.81 nmol/mL) (p=0.003). Vitamin C concentration and protein thiol levels did not differ between groups or environments. Mesocephalic dogs showed no difference in any of the markers when comparing the environments. Phospholipid membrane is the main mechanism affected by free radicals. This reaction results in the formation of malondialdehyde (MDA), an important indicator of oxidative stress. Elevated levels of lipid peroxidation, as measured by MDA concentrations, suggest that hospital stays can induce oxidative stress compared to home environments. On average, the dogs in the study showed higher values compared to the literature. In conclusion, malondialdehyde (MDA) concentration measured by the TBARS technique was higher in the hospital environment than in the home environment. This indicates that staying in a hospital environment induces the production of oxygen free radicals and oxidative stress. It is important to consider conducting oxidative stress assays in dogs in hospital settings, given that various variables in this environment can cause stress and subsequently lead to oxidative stress.

Keywords: malondialdehyde; hospitalization; internment.

3.2 INTRODUCTION

Oxidative stress results from an excess of reactive oxygen species (ROS), a reduction in antioxidant mechanisms, or both simultaneously. The role of oxidative stress in causing damage to the body is well recognized and contributes to a wide range of diseases including neoplasms, heart disease, trauma, and burns. Despite ROS being causative agents of cellular damage, under stable conditions they also play a regulatory role in cell function. An endogenous defense system modulates and controls the concentration of ROS, thereby preventing cellular damage. When this endogenous system fails or becomes overwhelmed, oxidative stress damage occurs (MCMICHAEL, 2007).

Stressful situations are linked to an increase in various markers indicating the presence of physiological changes in the body caused by such situations. One of these changes is the increased production of reactive oxygen species (ROS), which causes oxidative stress. Several stress factors have been studied, ranging from noise, training, immobilization, transportation, to restricted housing conditions (BEERDA et al., 1997; FERREIRA et al., 2014). Due to the short half-life of free radical molecules, they cannot be measured directly. In this context, malondialdehyde (MDA) is a product of lipid peroxidation used to indirectly assess ROS status. Some micronutrients, such as vitamins C and E, are non-enzymatic antioxidants that can be used to estimate oxidative stress levels (MAO; ZHANG; HUANG, 2014).

Recently, the popularity of brachycephalic dogs has increased. Genetic selection in these breeds has resulted in various anatomical sequelae that lead to airway obstruction in these dogs (ERJAVEC; VOVK; SVETE, 2021). The anatomical changes include narrowed nostrils, elongated soft palate, and hypoplastic trachea, which can lead to severe respiratory complications. Brachycephalic dogs surpass increased respiratory resistance. Respiratory resistance can be up to 16 times higher than in other breeds (DUPRÉ; HEIDENREICH, 2016; MEOLA, 2013; PHILLIPS, 2022).

Brachycephalic dogs experience episodes of apnea similar to humans with Obstructive Sleep Apnea Syndrome. These events result in increased generation of reactive oxygen species (ROS) and reactive nitrogen species, which can lead to endothelial dysfunction, vascular inflammation, atherosclerosis, and play a key role in the development of various heart diseases (ERJAVEC; VOVK; SVETE, 2021). There are several potential sources of reactive oxygen species (ROS) during apnea. Mitochondria are the main source of radicals during cellular respiration. During hypoxia, oxygen concentration is reduced, and radical production is increased due to excessive mitochondrial activation reduction. The main source of radical

creation refers to hypoxia and reperfusion; constant hypoxia followed by tissue reperfusion induces changes in energy metabolism (LAVIE, 2003).

In humans, recurrent episodes of airflow obstruction during sleep can lead to apnea and fluctuations in blood oxygen concentration. This constant hypoxemia and reoxygenation result in increased production of reactive oxygen species (ROS), leading to systemic oxidative stress, production of pro-inflammatory factors, metabolic dysregulation, and platelet aggregation. Systemic oxidative stress may play a key role in endothelial dysfunction and be a major factor in the increased cardiovascular risk in humans affected by sleep-disordered breathing (LAVALLE et al., 2024; MASTINO et al., 2023; STANEK; BROŻYNA-TKACZYK; MYŚLIŃSKI, 2021; YAMAUCHI et al., 2005).

Based on the above, the authors hypothesized that brachycephalic dogs may have higher oxidative stress generation compared to mesocephalic dogs, and that these changes may be exacerbated in a unfamiliar environment, such as a hospital setting. They also hypothesized that the timing of sample collection may influence the results, due to the circadian cycle's effect on oxidative stress generation. Therefore, the study aimed to evaluate oxidative stress in brachycephalic and mesocephalic dogs in both home and hospital environments, during nighttime and morning periods.

3.3 MATERIALS AND METHODS

This study was conducted from December 2023 to May 2024 at the Superintendência Unidade Hospitalar Veterinária Universitária (SUHVU) and Laboratory Block 3 of the Universidade Federal da Fronteira Sul - Campus Realeza. The research was approved by the Ethics Committee on Animal Use (CEUA) under protocol number 8451180923. All dog owners signed a consent form regarding the research and could withdraw at any time.

3.3.1 Animals

A total of 12 dogs were used for the study, based on a priori sample calculation with a power of 0.82 (effect size 0.8, α 0.05, and statistical power 80%) using G*Power software version 3.1.9.7. Dogs were randomly selected based on owners' adherence to the research. Screening involved physical examination, measurement of systolic blood pressure, echocardiogram, and electrocardiogram. Inclusion criteria specified that dogs should be healthy, aged between one and six years, and either brachycephalic or mesocephalic without abnormalities in the screening process. Exclusion criteria included any illness,

electrocardiographic or echocardiographic abnormalities, aggressive behavior, and screening process alterations. Selected brachycephalic dogs included three Pugs, two Shih Tzus, and the mesocephalic dogs included seven Mixed-Breed dogs. They had an average age of 4.5 ± 1.7 years and a body condition score of 5 ± 1.

3.3.2 Experimental Design

The dogs were divided into two groups: five brachycephalic dogs and seven mesocephalic dogs. The study involved collecting blood samples to assess oxidative stress during nighttime and morning periods in both home and hospital environments. Each dog participated in the study for three days within a week. On the first day, blood samples were collected in one of the environments, followed by 24 hours of rest. The study was then conducted in the opposite environment. The order of environments was randomly assigned for each dog.

3.3.3 Environments

During the experiment in the home environment, the dogs participated in their normal routines. Food availability was according to the owners' choice, and water was provided ad libitum. During the 24-hour period in the hospital environment, each dog was housed in a stainless-steel cage (120x80x60cm) in the ward room of the SUHVU. The room size was 33m². Temperature was controlled by air conditioning, maintained at 18ºC. Feeding during this period consisted of food provided by the owners, using the dog's own bowls when possible, and water was available ad libitum.

3.3.4 Sample Collection

The samples were collected at two time points: in the early evening at 18:00 and in the morning at 08:00. Blood samples were obtained by venipuncture of the jugular vein into 3ml tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. The blood samples were centrifuged at 3500 rotations per minute for 15 minutes. Plasma was separated using a micropipette and transferred into Eppendorf-type microtubes. The plasma samples were then frozen and stored in an ultra-freezer at -80ºC.

3.3.5 Oxidative Stress

The levels of lipid peroxidation in the plasma were measured using the TBARS technique, as described by (LAPENNA et al., 2001), involving the reaction of thiobarbituric acid with malondialdehyde, which forms chromophores with maximum absorbance at 532 nm in the spectrophotometer. The measurement of vitamin C was carried out through the reaction with dinitrophenylhydrazine at 37ºC, and the product was read spectrophotometrically at 520 nm (GALLEY et al., 1996). The concentrations of protein thiols in the plasma were determined according to Ellman (1959), following the reaction with 5,5'-dithiobis (2-nitrobenzoic acid), and the color was measured in the spectrophotometer at 412 nm.

3.3.6 Statistical Analysis

The data were analyzed using jamovi® software (version 2.5.5). The Shapiro-Wilk test was used to identify the distribution of the variables. Parametric variables were presented as mean and standard deviation, while non-parametric variables were presented as median and interquartile ranges (25-75%). A Generalized Linear Model (GLM) was conducted through Omnibus ANOVA, followed by Bonferroni post hoc, which evaluated differences and interactions between the independent variables: brachycephalic and mesocephalic groups, home and hospital environments, night and morning collection, for the dependent variables lipid peroxidation by TBARS technique, protein thiols, and vitamin C. The significance level considered was $p \leq 0.05$.

3.4 RESULTS

3.4.1 Comparison between brachycephalic and mesocephalic dogs, home and hospital environments, and collection during the night and morning

Evaluating all the dogs involved in the research, an increase in lipid peroxidation was evidenced by the increase in malondialdehyde (MDA) concentration when the dogs spent the day in the hospital, presenting a concentration of 8.08 (4.02-14.9) nmol/mL, compared to the home environment, 6.04 (3.78-8.30) nmol/mL ($p=0.046$). Regarding racial conformation, the concentrations showed no differences, with brachycephalic dogs showing a concentration of 5.43 (3.97-8.69) nmol/mL and mesocephalic dogs 6.70 (3.88-16.8) nmol/mL ($p=0.064$). As for the time of collection, the concentrations also showed no differences, with the evening

collection showing a concentration of 6.70 (3.72-12.7) nmol/mL and the morning collection 5.65 (4.02-9.44) nmol/mL (p=0.371).

Protein thiol concentrations, when compared between different racial conformations, showed no difference, with brachycephalic dogs presenting a concentration of 2.27 (1.81-2.63) nmol/mL and mesocephalic dogs 2.04 (1.61-5.03) nmol/mL (p=0.147). The concentration in the hospital environment was 2.26 (1.64-3.41) nmol/mL and in the home environment 2.20 (1.60-3.15) nmol/mL (p=0.881). Regarding the time of collection, the evening concentration was 2.25 (1.60-4.63) nmol/mL, and in the morning, it was 2.21 (1.73-3.21) nmol/mL (p=0.205).

As for vitamin C, the concentrations showed no differences. Comparing racial conformations, brachycephalic dogs had a concentration of 10.1 (5.51-25.9) μg/mL and mesocephalic dogs 11.1 (6.68-23.6) μg/mL (p=0.919). Regarding environments, the concentration in the home environment was 10.9 (6.32-25.2) μg/mL and in the hospital environment 10.6 (8.26-22.6) μg/mL (p=0.683). In different collection times, the concentration at night was 13.7 (7.65-23.4) μg/mL, and in the morning 10.4 (6.32-26.2) μg/mL (p=0.524). There were no interactions between the groups of dogs, environments, and collection times for all the dependent variables evaluated (p>0.05).

3.4.2 Effect of environment and collection time on brachycephalic and mesocephalic dogs

Figure 7 – Effect of environment on lipid peroxidation levels by MDA concentrations assessed by TBARS, protein thiol levels and vitamin C concentration in brachycephalic dogs

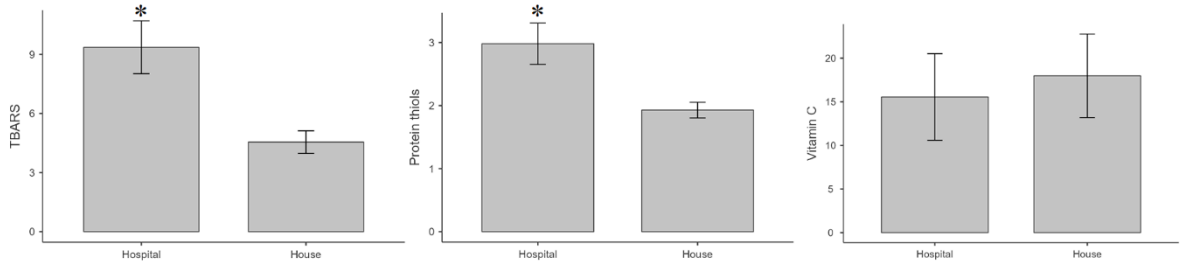
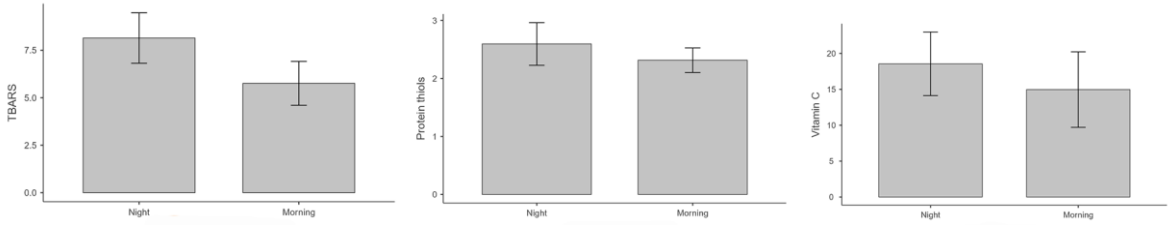


Figure 8 – Effect of collection time on lipid peroxidation levels by MDA concentrations assessed by TBARS, protein thiol levels and vitamin C concentration in brachycephalic dogs



Figures 7 and 8 summarize the effect of the environment and the effect of the collection time on lipid peroxidation levels through MDA concentration assessed by TBARS, protein thiol levels, and vitamin C concentration in brachycephalic dogs.

When evaluating the effect of environment and collection time on brachycephalic dogs, higher concentrations of MDA were observed in the hospital environment (9.36 ± 4.24 nmol/mL) compared to the home environment (4.55 ± 1.81 nmol/mL) (p=0.003). However, no significant differences were found between night collection (8.15 ± 4.20 nmol/mL) compared to morning collection (5.76 ± 3.65 nmol/mL) (p=0.108). There was no interaction between environments and collection time (p=0.432).

In terms of protein thiol concentration, brachycephalic dogs showed higher values in the hospital environment (2.98 ± 1.0 nmol/mL) compared to the home environment (1.93 ± 0.3 nmol/mL) (p=0.010). The concentrations from night (2.59 ± 1.1 nmol/mL) and morning (2.31 ± 0.6 nmol/mL) collections did not differ (p=0.446), and no interactions were identified between environments and collection times (p=0.456).

Regarding vitamin C concentration, brachycephalic dogs showed values that did not differ between the home environment (18.0 ± 15 μg/mL) and hospital environment (10.1 (8.34-17.3) μg/mL) (p=0.740). Similarly, there was no difference between collection times; night collection showed a value of 18.6 ± 14 μg/mL, while morning collection showed 8.58 (3.90-21.6) μg/mL (p=0.624). No interactions were identified between environments and collection times (p=0.558).

Figure 9 – Effect of environment on lipid peroxidation levels by MDA concentrations assessed by TBARS, protein thiol levels and vitamin C concentration in mesocephalic dogs

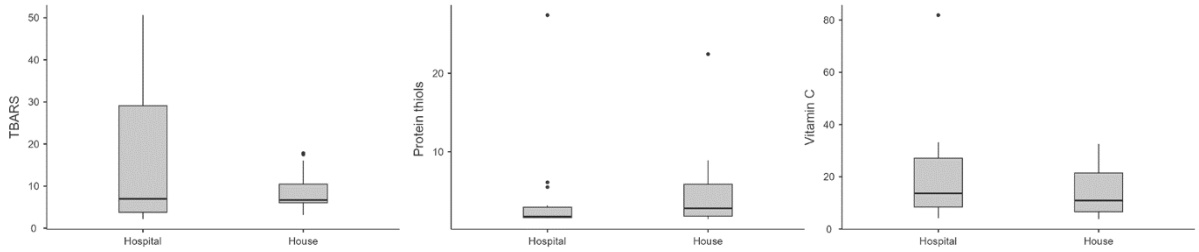
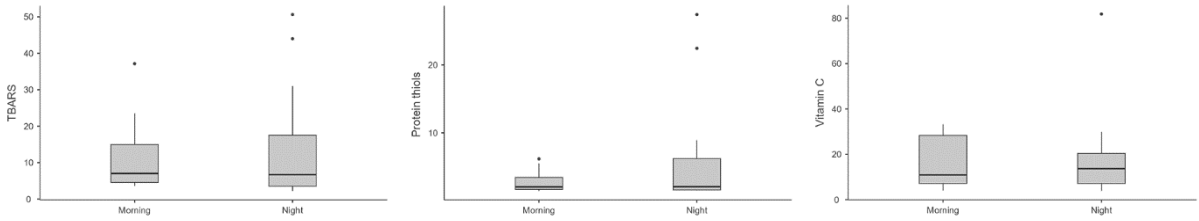


Figure 10 – Effect of collection time on lipid peroxidation levels by MDA concentrations assessed by TBARS, protein thiol levels and vitamin C concentration in mesocephalic dogs



Figures 9 and 10 summarize the effect of the environment and the effect of the collection time on lipid peroxidation levels through MDA concentration assessed by TBARS, protein thiol levels, and vitamin C concentration in mesocephalic dogs.

When evaluating the effect of environment and collection time on mesocephalic dogs, concentrations of MDA in the hospital environment (6.97 (3.77-29.1) nmol/mL) and home environment (6.70 (6.04-10.4) nmol/mL) did not differ (p=0.142). There were no differences between night collection (6.70 (3.50-17.5) nmol/mL) compared to morning collection (7.03 (4.55-15.0) nmol/mL) (p=0.544). No interaction was observed between environments and collection times (p=0.440).

Regarding protein thiol concentration, mesocephalic dogs showed values that did not differ between the hospital environment (1.73 (1.60-2.92) nmol/mL) and home environment

(2.79 (1.79-5.84) nmol/mL) (p=0.792). Similarly, there was no difference between night collection (2.08 (1.60-6.22) nmol/mL) and morning collection (2.04 (1.67-3.43) nmol/mL) (p=0.164). No interactions were identified between environments and collection times (p=0.937).

Regarding vitamin C concentration, mesocephalic dogs showed values that did not differ between the home environment (10.9 (6.60-21.4) μg/mL) and hospital environment (13.7 (8.42-27.1) μg/mL) (p=0.317). Similarly, there was no difference between night collection (13.7 (7.13-20.4) μg/mL) and morning collection (10.9 (7.13-28.4) μg/mL) (p=0.686). No interactions were identified between environments and collection times (p=0.729).

3.5 DISCUSSION

Concentrations of MDA in plasma were higher in dogs that spent the day in a hospital environment, indicating an increase in oxidative stress generation in this setting. This was observed in all dogs studied and specifically within the brachycephalic group. The phospholipidic membrane is the principal component affected by oxygen free radicals, which primarily target the polyunsaturated fatty acids in the membrane, resulting in disorganization and loss of cellular function. This reaction produces malondialdehyde (MDA) as a byproduct, which serves as an indicator of lipid peroxidation, generated by oxidative stress (PATTERSON; LEAKE, 1998; PEȘTEAN et al., 2024).

It is expected that in stressful situations, oxidative stress indicators also increase. Several factors causing stress have been studied and have been proven to result in oxidative stress. Dogs subjected to short-term transportation show increased MDA concentrations, indicating oxidative stress. They also showed increased total antioxidant capacity and 2,2-diphenyl-1-picryl-hydrazyl (DPPH), which are antioxidant mechanisms activated during oxidative stress processes (FERREIRA et al., 2014).

Increased oxidative stress is also evidenced in dogs undergoing surgical procedures. Both male and female dogs that were neutered showed a reduction in total antioxidant capacity and an increase in MDA concentration several days after the procedure (AENGWANICH et al., 2019; SAKUNDECH et al., 2020). Malondialdehyde serves as an excellent biomarker for identifying oxidative stress and has potential as a prognostic indicator for certain conditions such as neoplasms. However, it shows a low correlation with degenerative diseases like myxomatous mitral valve degeneration (SCHROERS et al., 2024; TOMSIČ et al., 2023).

Brachycephalic dogs, when spending the day in a hospital environment, showed an increase in protein thiols compared to the home environment. Glutathione is a tripeptide composed of cysteine, glycine, and glutamate, primarily stored and produced by the liver. Red blood cells contain an intracellular form of glutathione that serves as an antioxidant defense by neutralizing reactive oxygen species (ROS); this neutralization results in an oxidized form of glutathione. Through a recycling process involving enzymes and cofactors, oxidized glutathione is reduced back to its original form, reduced glutathione, which serves as an indicator of oxidative stress in the body (LUSHCHAK, 2012; MCMICHAEL, 2007). GSH is part of the group of protein thiols present in plasma. In our study, the increase in the concentration of protein thiols may indicate that other antioxidant systems were utilized to mitigate oxidative stress.

It is expected that under conditions of oxidative stress, the mechanisms responsible for antioxidation processes are utilized and experience a reduction in their concentrations. Dogs affected by visceral leishmaniasis, despite showing an increase in oxidative stress indicators such as MDA, did not exhibit a reduction in GSH concentrations in plasma and erythrocytes (BILDIK et al., 2004). Similarly, dogs presenting with anemia and chronic kidney disease at various stages did not show differences in erythrocyte GSH concentrations, despite having increased MDA concentrations in plasma (KOGIKA et al., 2015). However, dogs affected by non-hemolytic anemia showed a reduction in plasma GSH concentration compared to healthy dogs (WOOLCOCK et al., 2020). In our study, despite dogs showing an increase in MDA concentration in the hospital environment, indicating oxidative stress, we did not observe a reduction in GSH concentration. Instead, there was an increase in the concentration of GSH, which suggests that other antioxidant mechanisms were trying to contain oxidative stress.

Vitamin C, or ascorbic acid, is the first line of antioxidant defense in the body, acting as a potent antioxidant to prevent oxidative stress (PORATO et al., 2023). Being a potent antioxidant and one of the first lines of defense, dogs experiencing increased oxidative stress typically show a reduction in vitamin C concentration. This is evidenced in dogs with skin diseases, where an increase in MDA concentration correlates with a decrease in vitamin C concentrations (BEIGH et al., 2014b). Similarly, dogs affected by zinc-responsive dermatosis also showed a reduction in vitamin C concentration (BEIGH et al., 2014a). Moreover, in the presence of infectious diseases associated with high levels of oxidative stress, a reduction in vitamin C concentration is observed compared to healthy dogs (BILDIK et al., 2004). In our study, vitamin C concentrations did not show differences between groups with different breed conformations, environments, or collection times.

The study has some limitations, with the main challenge being the lack of standardization of airway obstruction severity in brachycephalic dogs. This inconsistency hinders the formation of a completely homogeneous group and the achievement of comparable and precise results among different subjects and study groups. It was not possible to perform all the desired oxidative stress analyses due to the loss of erythrocyte samples, as well as the evaluation of the enzymatic capacity of the antioxidant system, which could have provided a clearer understanding of the oxidative status of the dogs. Another consideration is the sample size, which despite being calculated a priori, comprises a small number of animals. It is suggested that further research be conducted with better standardization of breed conformation and a larger number of animals to more accurately identify changes in the oxidative status of dogs.

3.6 CONCLUSION

The results of our study indicate that the hospital environment can induce oxidative stress in dogs. When all the dogs evaluated in the study, regardless of breed conformation, spent the day in the hospital environment, an increase in MDA concentration was observed, which was not seen in the home environment. Additionally, an increase in the same indicator was identified in brachycephalic dogs, along with an increase in protein thiol levels in the hospital environment. However, breed conformation and the timing of sample collection do not seem to influence the generation of oxidative stress in dogs.

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