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ANIMAL SCIENCE

Heart rate variability and vasovagal tone index in brachycephalic dogs

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Abstract: Brachycephalic breeds of dogs, most of which show signs of the brachycephalic syndrome may have greater parasympathetic stimulation than other breeds, leading to higher values of heart rate variability and vagal tone index. The aim of this study was to establish a computerized electrocardiographic study and an assessment of the vagus sympathetic balance through heart rate variability and vagal tone index of five brachycephalic breeds compared to mesocephalic dogs. Sixty dogs were used, divided into groups made up of Boxers, English Bulldogs, French Bulldogs, Pugs, Shih-Tzu and no defined breed mesocephalic dogs. Statistical analysis was carried out using the Shapiro-Wilk test, Kruskal-Wallis and Dunn's test or ANOVA and Bonferroni ($p<0.05$). In the evaluation of vagal sympathetic balance among all the dogs, there was a negative correlation between heart rate and HRV 10RR ($r = -0.7678$; $p < 0.0001$), HRV 20RR ($r = -0.8548$, $p < 0.0001$) and VVTI ($r = -0.2770$; $p = 0.0321$). It can therefore be concluded that the dog's breed and morphology did not alter its electrocardiographic parameters or heart rate variability. The vagal tone index, which in other studies differed in brachycephalic dogs, showed no difference when compared separately in brachycephalic breeds.

Key words: vagal stimulation, racial pattern, electrocardiogram, autonomic nervous system.

INTRODUCTION

The study of heart rate variability (HRV) and the vasovagal tone index (VVTI) are non-invasive methods that assess the activity of the autonomic nervous system on the sinoatrial node (Baisan et al. 2021). As a result, an increase in the tone of the sympathetic autonomic nervous system is accompanied by a decrease in HRV and VVTI values, while an increase in parasympathetic activity and tone leads to higher HRV and VVTI values (Bogucki & Noszczyk-Nowak 2015).

Brachycephalic dogs, most of which have brachycephalic syndrome, can have a longer inspiratory duration and show a greater respiratory influence on the autonomic nervous system, thus altering their HRV (Silva et al. 2022). In addition, it is reported that brachycephalic breeds may have a more exaggerated respiratory sinus arrhythmia than other breeds (Tilley 1992).

There are few studies on HRV in dog breeds and the vast majority of studies are on just one breed. As in the study by Häggström et al. (1992) which analyzed HRV in the breed Cavalier King Charles Spaniel with endocardiosis and in the breed Doberman Pinscher with dilated cardiomyopathy (Calvert & Jacobs 2000).

Brachycephalic syndrome, also known as upper airway obstruction syndrome, is a respiratory disorder related to the shape of the face (Mitze et al. 2022). The shortening of the skull not associated with a proportional reduction in soft tissue results in deformation of the upper respiratory tract. If the nostrils, mucous membranes of the nasal conchae, soft palate and even the tongue are not reduced, the dimensions become incongruous and the lumen of the upper respiratory tract narrows. Stenotic nostrils, displacement and hyperplasia of the soft palate and relative macroglossia are the most common alterations (Ekenstedt et al. 2020).

An increase in airflow resistance occurs due to the morphological changes in brachycephalic animals, which results in hypoxic vasoconstriction and increased pulmonary pressure (Canola et al. 2018). Signs commonly observed in the physical examination of these animals are exercise intolerance, auscultation of rales or stridor, snoring and elaborate breathing when the animal is sleeping or playing. The severity of respiratory distress can be determined by the color of the mucous membranes, capillary refill time and posture (Meola 2013). As a result of negative intrathoracic pressure and an increase in inspiratory effort, gastrointestinal signs such as dysphagia, regurgitation and vomiting can also be reported (Dupré & Heidenreich 2016).

Anamnesis, clinical assessment and diagnostic imaging determine the diagnosis of brachycephalic syndrome. Radiography, fluoroscopy and computed tomography can be performed on the cervical and thoracic regions, while endoscopy is a method aimed at examining the upper airways (Dupré & Heidenreich 2016).

The electrocardiographic test evaluates the electrical functioning of the heart, producing a tracing that reflects the health of the heart. In order to accurately assess an ECG, several factors are taken into account. One of these aspects is heart rate variability (HRV), which is a variation between an individual's normal heart rate represented by an average value (Chuduc et al. 2013).

The wide range of possible uses, low cost of the technique and ease of obtaining the necessary data make HRV a good option for determining sympathovagal alterations (Vanderlei et al. 2009). Thus, an increase in the tone of the sympathetic nervous system is accompanied by a decrease in HRV values, while an increase in the tone of the parasympathetic nervous system results in an increase in HRV values (Baisan et al. 2021).

Autonomic balance indices can be obtained using different techniques, mainly categorized into the time domain and the frequency domain (Zupan et al. 2016). Frequency domain analysis transforms the variability of intervals between cardiac cycles into two types of frequency bands. High frequency waves represent markers of parasympathetic activity, while low frequency waves represent markers of sympathetic and parasympathetic activity (Bidoli et al. 2022). The time domain mainly involves an index obtained from the square root of the mean of intervals between successive cycles, giving rise to the RMSSD. It is one of the main indicators of variations related to parasympathetic activation (Zupan et al. 2016).

Due to the lack of studies using brachycephalic breeds, the aim of this study is to carry out a computerized electrocardiographic study and apply the HRV (Carareto et al. 2007) and VVTI (Doxey & Boswood 2004) indices to five different brachycephalic breeds and compare them with mesocephalic dogs.

MATERIALS AND METHODS

Animals

The animals were screened by clinical examination. The body condition score (BCS) was classified according to Laflamme (1997) on a scale of one to nine. All the dogs were healthy on physical examination. Exclusion criteria for the study were dogs under one year old and over eight years old, with chronic diseases or undergoing drug treatment. Dogs outside the breed standard were discarded.

The study was approved by the Ethics Committee for the Use of Animals at Universidade Vila Velha (CEUA - UVV) under protocol number 289-2013. All the owners agreed to take part in the project by signing an informed consent form.

Experimental Design

The procedures in this study were carried out at the dog's home in the presence of the owner. The data was collected and analyzed by a single evaluator for all the dogs in the experiment. In all groups, respiratory rate (RR), heart rate (HR), systolic blood pressure (SBP), Stop measurement and electrocardiography were determined. All the evaluations followed the same order: measurement of RR, HR, SBP, electrocardiography and finally measurement of the Stop.

Breed groups

A total of 60 adult dogs were assessed, divided into groups according to their breed. The breeds studied were the Boxer, the English Bulldog, the French Bulldog, the Pug, the Shih-Tzu and the mesocephalic medium-sized no breed defined (SRD, in portuguese sem raça definida). A total of 10 dogs were used in each group, with no distinction or sex ratio between them.

Evaluations

To measure RR, respiratory movements per minute (mpm) were counted by visual inspection, without manipulation or with little restraint. HR was determined by counting heartbeats per minute (bpm) by auscultating the dog's heart.

Before measuring SBP, the appropriate neonatal latex cuff (Dixtal® - United States) was determined for the dog being assessed. The animal was placed in the right lateral decubitus position to measure SBP. A vascular Doppler device (Parks® model 841 tablet - Oregon - United States) connected to headphones was used. Five SBP measurements were taken, where the values could not vary by more than 10 mmHg. The borderline values were discarded and then the arithmetic mean was calculated with the other values to determine the SBP of the evaluated dog.

For the computerized electrocardiogram, the animals were positioned in the right lateral decubitus position and the electrodes of the multichannel digital electrocardiograph (TEB® ECGPC VET - São Paulo - Brazil) were positioned according to the recommendations of Tilley (1992), recording the DII bipolar lead. For electrical conduction, 70% alcohol was applied to the skin at the site of each electrode. The electrocardiographic test was recorded for two minutes. To assess heart rhythm, the values of 20 consecutive RR intervals were measured after one minute and thirty seconds of recording. The presence of respiratory sinus arrhythmia (RSA) was determined when there was a variation of more than 10% between consecutive RR intervals (Tilley 1992).

Two formulas were used to assess vagus sympathetic balance using RR interval measurements. To assess heart rate variability (HRV 10 RR), the formula used by Carareto et al. (2007) and its modified form using 20 RR intervals (HRV 20 RR) were used. To obtain the vasovagal tone index (VVTI), the formula described by Doxey & Boswood (2004) was used, based on the measurement of 20 consecutive RR intervals.

A centimeter tape measure was used to measure from the tip of the snout to the stop. The Stop is the concavity in the profile of the muzzle where the straight plane of the muzzle is deviated. The Stop can be accentuated or discreet according to the breed's morphology (Bell et al. 2012). In this study, this measurement from the muzzle to the stop is simply referred to as the "stop" for better understanding.

For a clinical assessment of signs of brachycephalic syndrome, the presence of nostril stenosis and respiratory noise were observed in the animals evaluated.

Statistical Analysis

Once the study had been carried out, the data for all the variables was assessed for normality using the Shapiro-Wilk test. For variables with a non-parametric distribution, the Kruskal-Wallis test was used, followed by Dunn's post-test for comparisons between groups. Variables with a normal distribution were analyzed using Analysis of Variance (ANOVA) followed by the Bonferroni post-test for comparisons between groups. Spearman's correlation was used to determine the correlation between Stop, RR and HR with HRV 10RR, HRV 20RR and VVTI. The data obtained in the experiment was considered significant when $p<0.05$. Descriptive statistics were used for the qualitative variables.

RESULTS

Among the dogs without a defined breed, nine were female and one was male, with an average age of 3.8 ± 1.9 years, an average weight of 14.6 ± 6.0 kg and an average body condition score (BCS) of 5.1 ± 0.5. Among the Boxer breed, five were females and five were males, with an average age of 4.6 ± 3.0 years, an average weight of 27.0 ± 5.9 kg and an average BCS of 5.5 ± 1.0. Among the English Bulldogs, there were seven females and three males, with an average age of 3.0 ± 1.3 years, an average weight of 24.4 ± 3.5 kg and an average ECC of 5.8 ± 1.4. Among the French Bulldogs, eight were females and two were males, with an average age of 2.7 ± 1.7 years, an average weight of 10.12 ± 1.7 kg and an average ECC of 4.7 ± 0.5. Among the Pugs, six were females and four were males, with an average age of 5.8 ± 1.7 years, an average weight of 8.9 ± 1.4 kg and an average ECC of 5.4 ± 0.8. Among the Shih-Tzus, seven were females and three were males, with an average age of 3.5 ± 1.5 years, an average weight of 6.0 ± 1.2 years and an average ECC of 5.2 ± 0.6.

The presence of nostril stenosis and respiratory noise were not observed in the SRD group. The Boxer group showed 10% nostril stenosis and no cases of respiratory noise. In the English Bulldog group, there was evidence of 80% nostril stenosis and 90% respiratory noise. In the French Bulldog group, 90% of nostril stenosis and 60% of respiratory noise and in the Shih-Tzu group, 50% of nostril stenosis and 20% of respiratory noise. In the Pug group, nostril stenosis and respiratory noise were found in all the dogs evaluated.

Non-parametric distribution was observed for the values of P wave duration ($p=0.0127$), PR interval ($p=0.0304$), QRS complex duration ($p=0.0034$), RR ($p<0.0001$), SBP ($p=0.01$), Stop ($p<0.0001$), 10 RR intervals (10RR) ($p<0.0001$), 20 RR intervals (20RR) ($p<0.0001$), HRV 10RR ($p=0.0008$) and VVTI ($p=0.0219$).

Normal distribution was observed for P wave amplitude ($p=0.5277$), R wave amplitude ($p=0.7234$), QT duration ($p=0.3469$), HR ($p=0.1317$) and 20RR HRV ($p=0.08985$).

In the computerized electrocardiographic study of the present study, the P wave duration, PR interval, QRS complex duration, P wave amplitude and R wave amplitude did not differ between the brachycephalic breeds when compared to the mesocephalic group of SRD dogs. Analysis of the QT interval showed differences between brachycephalic groups, in which the Boxer group had higher values compared to the English Bulldog, French Bulldog and Shih-Tzu groups, although no differences were found between brachycephalic and mesocephalic dogs. As for the amplitude of the T wave, the SRD group showed 60% biphasic waves and 40% less than a quarter of the R wave. The Boxer, French Bulldog, Pug and Shih-Tzu groups showed 70% biphasic waves and 30% less than a quarter of the R wave. The English Bulldog group showed 40% biphasic waves and 60% less than a quarter of the R wave.

Electrocardiography showed 40% sinus rhythm and 60% RSA in the SRD group. The Boxer and French Bulldog groups showed 50% sinus rhythm and 50% RSA. The English Bulldog group showed 60% sinus rhythm and 40% RSA, the Pug group 10% sinus rhythm and 90% RSA and the Shih-Tzu group 70% sinus rhythm and 30% RSA. Sinus Arrest was seen in three groups, SRD and French Bulldog with one case in each, and in the Pug group with four cases observed.

In this study, RF did not differ between the breeds when compared to the SRD group. SBP in the Boxer and English Bulldog groups was higher than in the SRD group. There was an increase in HR in the English Bulldog and French Bulldog groups compared to the SRD group. In the Stop assessment, there was a reduction in the English Bulldog, French Bulldog, Pug and Shih-Tzu groups when compared to the SRD group.

A comparison of the groups' 10RR HRV showed a reduction in the English Bulldog and French Bulldog groups compared to the SRD group. The 20RR HRV of the English Bulldog, French Bulldog and Shih-Tzu groups showed a reduction when compared to the SRD group. VVTI did not differ between the breeds when compared to the SRD group.

A comparison of the groups' 10RR HRV showed a reduction in the English Bulldog and French Bulldog groups compared to the SRD group. The 20RR HRV of the English Bulldog, French Bulldog and Shih-Tzu groups showed a reduction when compared to the SRD group. VVTI did not differ between the breeds when compared to the SRD group.

DISCUSSION

Dogs of the English Bulldog and Pug breeds showed the highest percentage of cases of nostril stenosis and respiratory noise in this study. The Pug was the breed in which nostril stenosis and respiratory noise were observed in all the animals evaluated. This data is in line with the study by Torrez & Hunt (2006), which suggests that the English Bulldog's congenital conformation for brachycephalic syndrome is more severe and that Pugs tend to suffer more from complications caused by the syndrome.

The evaluation of QT intervals showed differences between brachycephalic breed groups (p=0.002), with Boxers showing higher values compared to English Bulldogs, French Bulldogs and Shih-Tzu. However, there was no difference between the brachycephalic groups and the group of mesocephalic dogs. In addition, all the QT interval values found were within the normal range (Wolf et al. 2000).

Dogs affected by brachycephalic syndrome have increased intrathoracic pressure due to greater resistance during the passage of airflow in the upper respiratory tract, which can cause respiratory sinus arrhythmia (Doxey & Boswood 2004). In the present study, RSA was observed in a high percentage in almost all groups of dogs, including mesocephalic dogs. The Pug group was the group with the highest percentage of RSA affecting 90% of the dogs used in the study, corroborating the study by Torrez & Hunt (2006) which identified a greater tendency for Pug breed dogs to show complications and alterations resulting from the syndrome than other brachycephalic breeds.

The statistical difference found when comparing the SBP of the Boxer group and the English Bulldog group with the SRD group indicates median values within the parameters of low risk of damage to target organs, being classified as pre-hypertensive according to the work of Acierno et al. (2018).

The results showed that VVTI, an indicator of parasympathetic activity and heart rate variability in the time domain, did not differ between the brachycephalic breeds studied when compared to mesocephalic dogs. This result is contrary to the study carried out by Doxey & Boswood (2004), in which a significant difference was observed between the VVTI in different breeds, especially in brachycephalic breeds, which had a higher VVTI than the other breeds studied. According to the authors, the VVTI represents the HRV well due to the influence of breathing, in which brachycephalic dogs, because they have a longer inspiratory period, would have higher VVTI values, demonstrating the influence of breathing on the autonomic nervous system. However, the study included animals with heart disease, which have lower VVTI values when compared to patients in the early stages of the disease (Pereira et al. 2008, Rasmussen et al. 2012, Spier & Meurs 2004).

It is interesting to note that brachycephalic syndrome has different degrees of severity, causing different impacts on dogs. Brachycephalic syndrome can be classified into four grades from zero to three, with zero being less severe and three being more severe (Liu et al. 2015). The dogs in this study were not classified according to the severity of the brachycephalic syndrome, which is a limiting factor in the results.

In addition, the VVTI value found in this study in French Bulldogs was lower (6.7) than that found in the study by Trauffler et al. (2019), which established reference values (8.8) for this breed. However, according to Calvert & Jacobs (2000), the excess of respiratory sinus arrhythmia may have hindered the analysis in the time domain. The authors also suggest that techniques for analyzing HRV in dogs still have important limitations.

In this study, the dog's breed and morphology did not alter its electrocardiographic parameters or heart rate variability. The vagal tone index showed no difference when compared separately in brachycephalic breeds. The use of the formula proposed by Carareto et al. (2007), modified with 20 consecutive RR intervals, proved to be more sensitive in detecting heart rate variability than the original formula with 10 RR intervals. The Pugs evaluated in the study showed more clinical signs such as nostril stenosis, respiratory noise and respiratory sinus arrhythmia than the other breeds evaluated. This may suggest that the Pug tends to suffer more from the complications of brachycephalic syndrome.

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