Establishing reference standards for the vasovagal tonus index in a cohort of healthy French Bulldogs

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Summary

The vasovagal tonus index (VVTI), a time-domain indicator of heart rate variability, has been suggested as a diagnostic and prognostic tool in dogs with cardiac disease and heart failure. Brachycephalic breeds tend to have a higher VVTI than non-brachycephalic breeds.[11] The objective of this study was to obtain standard VVTI reference values from a representative cohort of healthy French Bulldogs based on a single ECG recording. A second aim of this study was to evaluate the practicability of a VVTI screening in the clinical routine by evaluating repeatability of the VVTI across five successive measurements. In order to determine baseline factors potentially influencing the VVTI, the impact of stress, activity and character as well as sex and neutering status were evaluated. Out of 73 French Bulldogs screened, 60 dogs were considered healthy based on their medical history, physical examination, blood pressure and ECG, and no evidence of congenital or acquired heart disease on echocardiographic examination. The VVTI was calculated based on the variance of 20 R-R intervals and the corresponding HR extrapolated to one minute from the same 20 R-R intervals. In addition, a maximum of five consecutive VVTI values were calculated based on 100 successive R-R intervals recorded from each dog. VVTI values ranged from 5.66 to 11.3 with a mean (standard deviation [SD]) of 8.82 (1.43); the corresponding HR ranged from 78 to 173 bpm with a mean (SD) of 120 (23) bpm. Importantly, VVTI and HR values were negatively correlated ($R = 0.689$; adjusted $R^2 = 0.466$), which must be considered for clinical interpretation of the VVTI. The repeatability across five successive sets of 20 heartbeats was shown (mean intra-individual variability of 6.1%). Stress significantly influenced the VVTI and HR ($p < 0.001$). The VVTI range established in this study may be used as reference to assess the HRV of presumably healthy brachycephalic dogs at routine health checks. Deviations from the reference may permit the clinician to adapt the schedule and focus of subsequent follow-up investigations.

Keywords: Brachycephalic, Dog, Heart Rate Variability, VVTI

Bestimmung der Referenzwerte des Vasovagalen Tonusindex bei gesunden französischen Bulldoggen

Der Vasovagale Tonusindex (VVTI) ist ein Zeitbereichsindikator für die Herzfrequenzvariabilität und wird als diagnostisches und prognostisches Instrument bei Hunden mit Herzerkrankungen und Herzinsuffizienz beschrieben. Brachyzephale Rassen haben tendenziell einen höheren VVTI als nicht-brachyzephale Rassen. [11]

Das Ziel dieser Studie war es, die VVTI-Referenzwerte von einer repräsentativen Kohorte gesunder französischer Bulldoggen mittels einer EKG-Aufzeichnung zu bestimmen. Ein zweites Ziel dieser Studie war es, die Praktikabilität eines VVTI-Screenings im klinischen Alltag zu evaluieren, indem die Wiederholbarkeit des VVTI über fünf aufeinanderfolgende Messungen untersucht wurde. Um die Basisfaktoren zu bestimmen, die möglicherweise den VVTI beeinflussen, wurden die Auswirkungen von Stress, Aktivität und Charakter sowie Geschlecht und Kastrationsstatus bewertet. Von 73 untersuchten französischen Bulldoggen galten 60 Hunde aufgrund ihrer Anamnese, körperlichen Untersuchung, ihres Blutdrucks und ihres EKGs als gesund, auch wurde bei der echokardiographischen Untersuchung keine angeborene oder erworbene Herzkrankheit festgestellt. Der VVTI wurde auf der Varianz von 20 R-R-Intervallen berechnet und der entsprechenden extrapolierten Herzfrequenz (HR). Zusätzlich wurde von jedem Hund maximal fünf aufeinanderfolgende VVTI-Werte basierend auf 100 aufeinanderfolgenden R-R-Intervallen berechnet.

Die VVTI-Werte lagen zwischen 5,66 und 11,3 mit einem Mittelwert (Standardabweichung [SD]) von 8,82 (1,43). Die entsprechende Herzfrequenz lag zwischen 78 und 173 Schlägen pro Minute mit einem Mittelwert (SD) von 120 (23) Schlägen pro Minute. Für die klinische Interpretation des VVTI muss die negative Korrelation von VVTI und HR ($R = 0{,}689$; angepasstes $R2 = 0{,}466$) berücksichtigt werden. Die Wiederholbarkeit konnte an fünf aufeinanderfolgende Sätze von

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20 Herzschlägen überprüft werden (mittlere intraindividuelle Variabilität von 6,1%). Stress beeinflusste den VVTI und die HR signifikant (p <0,001). Der in dieser Studie ermittelte VVTI-Bereich kann für die Beurteilung der Herzfrequenzvariabilität bei brachycephalen Rassen als Referenzwert während einer routinemäßigen Gesundheitsuntersuchung verwendet werden. Eine Abweichung von den Referenzwerten erlaubt es dem Kliniker Nachuntersuchungen anzupassen.

Schlüsselwörter: Brachyzephalie, Hund, Herzfrequenzvariabilität, VVTI

Introduction

The intrinsic heart rate (HR) is generated in the sinoatrial node, which is broadly under control of the autonomic nervous system, where parasympathetic and sympathetic activity constantly interact.[15,16] These complex interactions are reflected in variable R-R intervals between consecutive heart beats, which is referred to as heart rate variability (HRV). In dogs and humans, rapid changes in heart rate (HR) that occur on a beat-to-beat basis (referred to as high frequency variability) were shown to solely reflect parasympathetic tone,[2,24] whereas more slowly occurring changes (referred to as lower frequency HRV) result from the interaction of sympathetic and parasympathetic activity as well as from other influencing factors such as baroreflex-modulated blood pressure, stress, exercise, thermoregulation, the action of the renin-angiotensin system and the circadian biorhythm.[1,7] In conditions where the sympathetic nervous system is activated and vagal activity is reduced, such as in heart failure or under stressful conditions, an imbalance of the autonomic nervous system arises,[12] which can be non-invasively assessed by quantifying HRV. In humans, assessment of HRV has become an important risk assessment tool; reduced HRV being associated with progressive myocardial failure and worse outcome post-myocardial infarction.[4,20,27] Both time- and frequency-domain analyses can be used to assess HRV,[3,27] but many methods of analysis require electrocardiogram (ECG) recordings for periods of 24 hours or more.[7] In 1996, Häggström was the first to assess HRV in dogs using the so-called vasovagal tonus index (VVTI), which is a time-domain indicator of HRV obtained from ECG recordings.[13] As it is assessed over a short time period, it provides information about high frequency variations in HR. Since efferent vagal activity is a major contributor to the high frequency component under resting conditions,[2,17,24] the VVTI represents an index of vagal tone in dogs at rest. The study by Häggström suggested that the VVTI can be used as a tool supporting the diagnosis of cardiac decompensation in dogs with mitral insufficiency.[13] Since then, its role has been further investigated by additional research. The VVTI was reported to diminish as animals progress into heart failure and to show a trend towards an increase in animals responding to therapy.[5] The VVTI may serve as a diagnostic tool to assess severity of heart failure (although with low specificity) and as a prognostic indicator in dogs with dilated cardiomyopathy.[23] A more recent study suggested that the VVTI may be a potential predictor of cardiac and all-cause mortality.[18] These results lend further support for its purported value as a biomarker in veterinary practice. Importantly, the first study to evaluate the VVTI in different breeds of dogs reported a higher VVTI in brachycephalic compared to non-brachycephalic breeds.[11] To the authors' knowledge, no VVTI reference standards for healthy dogs of a representative brachycephalic breed have been reported so far. Reliable VVTI reference values from healthy dogs are however indispensable if this parameter is to be used for diagnostic and/or prognostic research and in clinical practice. The objective of this study was therefore to obtain VVTI reference standards in a cohort of healthy French Bulldogs as a representative brachycephalic breed. A second aim of this study was to evaluate the practicability of a VVTI screening in the clinical routine by evaluating repeatability across five successive VVTI measurements. Furthermore, in order to determine potential baseline factors influencing the VVTI, impacts of stress, activity and character as well as sex and neutering status on the VVTI were evaluated.

Materials and methods

Study design

This was a prospective observational single centre study conducted over five months with consecutive recruitment and a recruitment target of 60 dogs. It was conducted at the small animal university hospital of the Vetsuisse Faculty Bern, Switzerland, between May and September 2015. Approval by the cantonal animal research committee (KTV) (TVB BE 33/16) and informed owner consent were obtained.

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Study population

Dogs were randomly recruited from a privately owned, mainly FCI-accredited population to provide a comprehensive health check-up. Dogs were required to be healthy, which was determined based on their medical history, normal physical examination, ECG and echocardiography as well as blood pressure (BP) within the normal range. All dogs were required to be over seven months of age and no sex or weight limitations were imposed. In the event that rectal temperature was > 39 °C or the dog did not tolerate the key assessments, the dog was excluded.

Data acquisition and methods

All procedures were conducted using the same protocol and in the presence of the owner. The examinations were performed in the late morning or early afternoon under fasting conditions and without the use of sedation. Care was taken to eliminate extraneous noise or other environmental distractions, such as people entering the room. Five to 10 minutes were allowed for acclimatisation. Physical examination and auscultation were performed successively, followed by echocardiography or by ECG and BP measurement, adapted to the situation and performed in a manner to minimise stress. The dogs were gently placed in right lateral recumbency for echocardiography, ECG and BP measurements.

Screening questionnaire

In the absence of validated tools to appropriately reflect dog fitness, acute stress levels and general character, a simple questionnaire was devised for the purpose of this study. It was completed by the owner, took into account the dog's medical history and evaluated the following features: *activity level:* grade 1 (<1 hour of physical activity per day), grade 2 (1-2 hours of physical activity per day) and grade 3 (>2 hours of physical activity per day); *acute stress level:* tense versus relaxed and *general character type:* nervous/temperamental versus calm/not easily irritated. The absence of a validated questionnaire must be seen as a limitation of this study.

Physical examination

Signalment, history, general appearance, vital signs and a physical examination were obtained for each dog. The physical examination included cardiac/respiratory auscultation, assessment of heart/pulse rate, respiratory rate and pattern, abdominal palpation, evaluation of mucous membrane colour and capillary refill time, rectal temperature and body weight.

Echocardiography

Echocardiographic examinations were performed by the same board-certified cardiologist with a highframe rate ultrasound system (Aloka, Prosound 5α SV) equipped with a 5 MHz-probe that permitted simultaneous ECG recordings. Images and loops from standardized imaging planes[28] were digitally stored. Pulmonic flow velocity was measured by pulsed-wave Doppler and aortic flow velocity was measured by subcostal continuous wave Doppler. Peak velocities had to be below 2.2 m/s. Morphology of all four valves was evaluated with 2D imaging in each imaging plane that showed the valve. Mitral and tricuspid valves had to show complete closure without regurgitation jets to meet the inclusion criteria.

Systemic blood pressure

Blood pressure (BP) measurement was standardized for this study. BP was measured non-invasively either by oscillometry or by Doppler flow detection, depending on the dog's physical form. An oscillometric device (Cardell Model 9500 HD) with a distal hindlimb cuff was used for mean arterial blood pressure (MAP) measurement and a Doppler flow detection system (Doppler Flow Detector Model 811-B) with a distal forelimb cuff was used for systolic arterial blood pressure (systolic BP) measurement – following a published protocol.[6] BP device cuff size was determined based on the dog's leg shape (approximately 40% of circumference of the cuff site). The mean of three pressure measurements was calculated and taken further for analysis. BP within the normal range was defined as systolic BP ≤ 150 mmHg or MAP ≤ 115 mmHg, depending upon the method used.

Electrocardiography

The ECG recordings were performed with the dog placed in right lateral recumbency using a commercially available ECG unit (Schiller Type Cardiovit AT-101) and a standard six-lead recording system (limb leads I, II, III, aVR, aVL, aVF). Electrodes were attached to the skin with atraumatic clips on each limb. Alcohol was used to improve electrical contact. The ECG was recorded for one minute to get the dog acclimatized to the clips. To begin, recordings were obtained from all six leads for approximately 10 seconds. Approximately five-minute recordings from leads I, II and III were subsequently obtained to assess a continuous run of sinus rhythm for at least 100 consecutive R-R intervals. All measurements were derived from lead II recordings. In order to obtain the accurate HR corresponding to each VVTI calculation, HR was calculated in parallel to each VVTI calculation by extrapolating 20 heartbeats to one minute using the formula 20 * 60000/[Z1+Z2+(…) +Z20 (ms)] (bpm). The paper speed used was 50 mm/s.

Calculation of heart rate variability using the VVTI

HRV was defined as the vasovagal tonus index (VVTI).[13] One hundred consecutive PQRST complexes with sinus rhythm from the ECG recording were obtained in order to calculate five successive VVTI values. R-R interval durations were measured with a ruler to the nearest half of a millimeter (equivalent to the nearest 10 milliseconds). The VVTI was calculated as the natural logarithm of the variance of the R-R intervals using the formula VVTI = LN [VAR(Z1-Z20) (ms)].[13] Five successive VVTI values were calculated from a maximum of 100 consecutive R-R intervals (VVTI1 to VVTI5) in order to evaluate repeatability of the results using the intra-class coefficient of variation.

Statistical analysis

All data were statistically analysed using SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 24 Armonk, NY: IBM Corp.). Boxplots of obtained VVTI values were produced using GNU R (R Core Team 2018) and package ggplot2 (Wickham H 2016).

VVTI and HR values of the first measurement (VVTI1 and HR1), blood pressure (BP), age, body weight and body temperature were statistically described by mean, minimum, maximum, 25, 50 and 75 percentiles and standard deviation (SD). Ninety-five percent confidence intervals (CIs) as well as 95% prediction intervals (PIs) were calculated. Parameters were also analysed classified by sex and neutering status (male, neutered male, female, neutered female). The Kolmogorov-Smirnov test was used to test for normality.

To assess repeatability of VVTI results, intra-individual variability was estimated for all dogs based on a maximum of five successive VVTI values. The mean CV was then calculated for the entire cohort (n = 60). Inter-individual variability estimation was based on the first VVTI measurement in order to assess fluctuations within the study population. Pearson's correlation coefficient was used to assess relationships between VVTI1 and HR1 and multiple linear regression analysis was used to confirm the relationship between HR1 and VVTI1.

All distinct VVTI values obtained (n = 238) and their corresponding HR were pragmatically categorized by HR intervals of 10 bpm (obtaining HR categories ranging from 80 to 210 bpm) and statistically described.

Further, in order to assess the impact of the baseline factors activity, stress, character, sex and neutering status on VVTI and HR, the dogs were accordingly classified into groups and an unpaired t-test or a one-way ANOVA test were used to compare VVTI1 and HR1 between groups. Box and whisker plots were used to illustrate the results. For all statistical analyses a p-value $< 5\%$ ($p < 0.05$) was considered significant.

Results

Study population

A total of 73 French Bulldogs were screened. Sixty dogs met the criteria for inclusion in the study. Thirteen dogs were excluded for the following reasons: ectopic atrial rhythm (n = 3), AV-block (n = 2), atrial septum defect (n = 1), pulmonary (n = 3), aortic (n = 1), tricuspid (n = 2) and mitral valve insufficiencies (n = 2) (one dog presenting with both minimal mitral- and tricuspid regurgitation). These findings appear representative for this breed, although scientific literature is scarce on the prevalence of cardiac disorders in French Bulldogs.[14,21,22] Medical records included cases of traumatic injury (fracture of fore- or hindlimb), orthopedic disease (hip dysplasia, patella luxation), surgical intervention (intestinal foreign body, disc herniation), allergy or ocular disease (cornea lesion). Previous surgical treatment of brachycephalic syndrome (more than six months previously) was reported for five dogs. Vertebral malformations were reported for three dogs. No additional radiographs were performed for the purpose of this study. Dogs were not receiving any medical therapy at the time of the study.

In three dogs, mean arterial pressure was less than 80 mmHg (77, 77 and 74 mm Hg, respectively). Since systolic BP measurements, physical, ECG and echocardiographic examinations were normal, these dogs were not excluded from the study. In the remaining dogs, BP measurements were within the normal range.

Twenty-nine dogs were male (nine neutered) and 31 female (14 neutered) with a mean age of 3.53 years (range, seven months to 11.8 years). Mean body weight was 12.2 kg (range, 7.6 to 16.6 kg). Mean body temperature was 38.5 °C (range, 37.8 to 39 °C).

Study results

VVTI reference values

For the first measurement in the analysed cohort (n = 60), the VVTI ranged from 5.66 to 11.3 (mean, 8.82) and the corresponding HR ranged from 78 bpm to 173 bpm (mean, 120 bpm). VVTI and HR values obtained are presented as reference values with corresponding 95% CIs and PIs in Table 1. It was not possible to obtain 100 consecutive R-R intervals for several un-

Table 1: VVTI and HR references values (minimum, maximum and mean values with standard deviation and corresponding 95% confidence and prediction intervals) obtained from 60 healthy French Bulldogs are displayed.

	N	Range	Mean	SD	95% CIs	95% PIs
VVTI	60	5.66 - 11.3	8.82	1.43	8.46 – 9.18	8.39 – 9.25
HR (bpm)	60	78 - 173	119.6	23.4	113.6 – 125.5	112.6 – 126.7

CIs, confidence intervals; HR, heart rate; N, number of dogs; PIs, prediction intervals; SD, standard deviation; VVTI, vasovagal tonus index

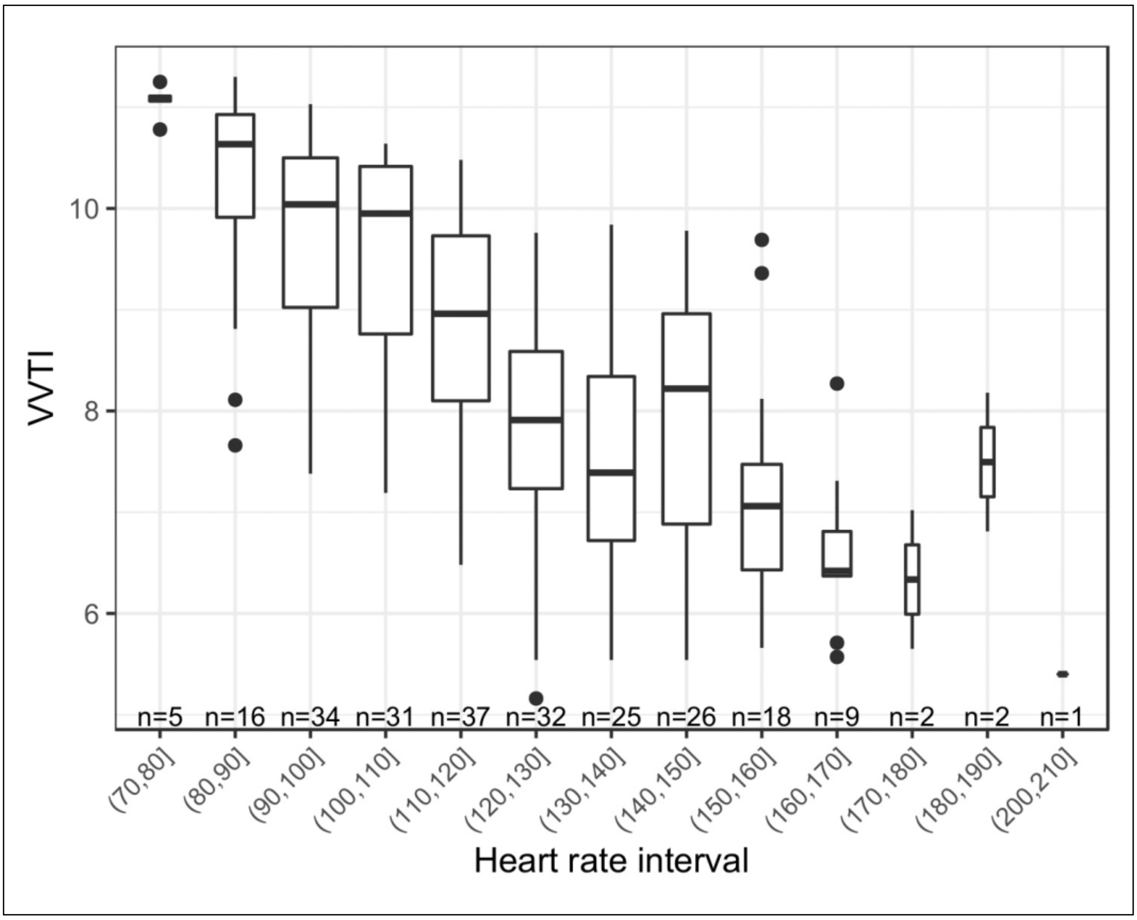

Figure 1: All VVTI values obtained are descriptively illustrated by heart rate categories, each spanning 10 bpm. The negative correlation between VVTI and HR values is highlighted. It is evident that within one HR category of 10 bpm, high variability of the VVTI can be observed and that several HR categories cover the same broad VVTI ranges.

cooperative dogs and, consequently, five VVTI values could only be obtained in a smaller cohort (n = 31). The VVTI and HR data obtained were however similar in the smaller cohort of 31 dogs compared to the overall cohort: for the first measurement, the VVTI ranged from 5.66 to 10.86 (mean 8.49) and HR ranged from 88 bpm to 162 bpm (mean, 124 bpm).

The assumption of normality was confirmed using Kolmogorov-Smirnov test with p-values ranging from 0.2 to 0.97 for all tested parameters.

Pearson's correlation coefficient showed a statistically significant negative correlation between VVTI1 and HR1 ($p < 0.01$). To confirm the negative relationship between HR1 and VVTI1, a multiple linear regression analysis was performed. Using HR1 as predictor, an R value of 0.689 and adjusted R^2 of 0.466 were obtained. The negative correlation between VVTI and HR is highlighted in Figure 1, where the VVTI data are descriptively illustrated by heart rate categories, spanning 10 bpm each. Multiple observations per dog were integrated into this analysis (n = 238).

VVTI and HR analysed by gender

For the first measurement in the female cohort, VVTI values ranged from 5.66 to 11.25 (mean, 8.5) and corresponding HRs ranged from 80 to 173 bpm (mean, 124). For the male cohort, VVTI values ranged from 6.74 to 11.3 (mean, 9.16) and HRs from 78 to 154 bpm (mean, 115 bpm). Corresponding 95% CIs/PIs are displayed in Table 2. The one-way ANOVA test comparing the VVTI and HR between female, female neutered, male and male castrated dogs showed no statistically significant differences between the four groups ($p > 0.05$).

Table 2: VVTI and HR reference values (minimum, maximum and mean values with standard deviation and corresponding 95% confidence and prediction intervals) are displayed separately for the female (n = 31) and male (n = 29) cohort of French Bulldogs.

	Gender (N)	Range	Mean	SD	95% CIs	95% PIs
VVTI	Female (31)	5.66 - 11.25	8.5	1.51	7.97 – 9.03	7.81 – 9.19
HR (bpm)	Female (31)	80 - 173	123.5	23.6	115.2 – 131.8	112.8 – 134.2
VVTI	Male (29)	6.74 – 11.3	9.16	1.27	8.69 – 9.62	8.56 – 9.76
HR (bpm)	Male (29)	78 - 154	115.3	22.4	107.2 – 123.5	104.7 – 126

CIs, confidence intervals; HR, heart rate; N, number of dogs; PIs, prediction intervals; SD, standard deviation; VVTI, vasovagal tonus index

Intra-individual variability
Coefficients of variation (CV) of the VVTI were calculated for each dog in order to estimate intra-individual variation based on a maximum of five successive measurements, i.e. to assess repeatability of the VVTI. CVs ranged from 0.6 to 16.9% with a mean of 6.1% (95% CIs of 5.0 to 7.1%) in the analysed cohort (n = 60). In the smaller cohort of dogs with five available VVTI values (n = 31), CVs ranged from 0.6 to 16.9% with a mean of 6.0% (95% CIs of 4.5 to 7.4%), confirming the results obtained for the entire cohort.

Inter-individual variability
In order to evaluate between-dog comparisons, the coefficient of variation for the first VVTI measurement was estimated for the entire cohort: a CV of 16.2% (n = 60). In the smaller cohort of dogs with five VVTI values available, the result was similar: a CV of 17% (n = 31).

Evaluation of influencing factors
In order to assess the impact of the baseline characteristics stress, character and activity on the VVTI and HR, the dogs were further classified by *acute stress level* [subgroups tense (n = 35) versus relaxed (n = 15)], *type of character* [subgroups nervous (n = 23) versus calm (n = 37)] and *level of activity* [class 1 (n = 3) versus class 2 (n = 45) versus class 3 (n = 12)]. The unpaired t-test yielded the following results: for the baseline characteristic '*acute stress level*', relaxed dogs showed a statistically significantly higher VVTI and a lower HR than tense dogs ($p < 0.001$). Statistically significant differences for the VVTI and HR were neither found for the baseline characteristic '*type of character*' comparing the subgroups nervous versus calm ($p > 0.05$) nor for the characteristic '*activity level*' comparing three different fitness grades ($p > 0.05$).

Discussion

This study aimed to establish VVTI reference standards for the healthy French Bulldog based on ECG recordings obtained at a single time point. Results of a previous study comparing the VVTI of brachycephalic with non-brachycephalic breeds suggest a higher VVTI in the former (mean VVTI of 8.46) compared to non-brachycephalic breeds (mean VVTI of 7.53),[11] which may require differentiating between these two. Reasons for a higher VVTI in brachycephalic dogs remain unclear, but may in part be explained by the greater inspiratory effort dictated by morphology. The dogs included in our study are considered representative of a healthy French Bulldog population seven months of age and older, as all dogs were randomly recruited from a presumably healthy, privately owned, mainly FCI-accredited population and no gender or weight limitations were imposed. All dogs were considered healthy based on their medical records and concurrent clinical examinations. Several dogs showed clinical signs associated with brachycephalic syndrome. However, the present study was not designed to assess brachycephaly and therefore no conclusions can be drawn in this respect.

VVTI values obtained in our study are in agreement with previously reported results by Doxey and Boswood, who investigated different breeds of dogs.[11] It should be noted however that results from their study ought to be interpreted carefully as also dogs with heart disease (including heart failure) were included. As previous studies have indicated that the VVTI is significantly lower in dogs with heart failure compared to dogs with earlier stages of cardiac disease[8,9,11,13,19,23,25,26], values excluding the dogs with heart failure enable a more meaningful comparison: when dogs with heart failure are excluded from that study data, mean VVTI values were 8.82 for Boxers, 8.56 for Bulldogs and 8.86 for Cavalier King Charles Spaniels. Besides good concordance with the findings presented here, these data indicate that the VVTI values established in our study may be extrapolatable to other brachycephalic breeds.

The reference values established in this study permit clinicians to determine whether a VVTI value obtained from a presumably healthy brachycephalic dog at their veterinary practice undergoing a routine health check is within the normal range. Deviations from the reference may be used to adapt the schedule and focus of subsequent follow-up investigations.

Since the HR and VVTI are significantly negatively correlated, all obtained VVTI values (n = 238, multiple observations per dog) were further analysed by HR categories, spanning 10 bpm each. Figure 1 highlights the negative correlation between the VVTI and the HR. It is evident that, within one HR category, a high variability of the VVTI can be observed and that several HR categories cover the same broad VVTI ranges. These findings imply that the 'normal' VVTI range, as observed in the healthy dogs in this study, is relatively broad and, importantly, that HR and VVTI must be considered jointly in making clinical judgements. Results obtained for the HR categories below 100 bpm and above 150 bpm must be interpreted with caution due to the small number of observations obtained.

As concurrent levels of sympathetic tone may influence HRV, we compared VVTI and HR values between two subgroups of stress level and observed that relaxed dogs had a significantly higher VVTI and lower HR compared to tense dogs. This may be explained by the high correlation observed between the VVTI and the HR and reinforces the need to minimise stress before and during ECG recordings.

As physical activity has been described as another influencing factor of canine HRV,[10] we also compared three groups of activity levels (physical activity grades 1 to 3). No statistically significant differences could be detected on comparing HR and VVTI values between the three different subgroups. Therefore, these findings indicate that VVTI values are not markedly influenced by the level of physical activity. Nevertheless, use of a pragmatic questionnaire to elicit activity levels together with the limited sample size of the group with lowest physical activity ($n = 3$) warrants careful interpretation of these particular findings.

As this study provides a reference range for healthy dogs, further investigations on the VVTI in a sample of dogs with cardiac disease will be needed in order to characterize VVTI thresholds indicative or predictive of cardiac decompensation or response to therapy.

Addition of a 24 hours ECG analysis could have been useful to calculate other indices of HRV that provide information about lower frequency R-R variations influenced by net autonomic control as well as additional mechanisms such as contributions from the renin-angiotensin system and the circadian biorhythm.[1,7] In contrast, short-period VVTI assessment reflects high frequency variations of the HR and only accounts for HRV related to parasympathetic inputs.[2,24] While long-term ECG analysis was outside the scope of this study, the methods and results presented are more applicable to routine clinical investigations.

Finally, as regards the practicability of a VVTI screening in daily clinical practice, we show repeatability of the VVTI across five successive sets of 20 heartbeats. A mean intra-individual CV of 6.1% was observed in the whole cohort. Assuming that a variation of less than 10% can be tolerated, we consider that one single measurement of the VVTI obtained from 20 heartbeats is sufficiently reliable for clinical use.

Conclusion

This study has established VVTI reference values for a healthy brachycephalic breed of dog. These VVTI values cover a relatively broad range and, importantly, they negatively correlate with HR, which needs to be considered for clinical interpretation of the VVTI. Stress significantly affected VVTI and HR values, highlighting the need to minimize its impact before and during ECG recordings. One VVTI value obtained from 20 heartbeats is considered sufficiently reliable for clinical use based on a mean intra-individual CV of 6.1% in the whole cohort. The VVTI range established in this study may be used as a reference to assess the HRV of presumably healthy brachycephalic dogs at routine health checks. Deviations from the reference may permit the clinician to adapt schedule and focus of subsequent follow-up investigations.

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Détermination des valeurs de référence de l'indice de tonicité vaso-vagal chez des bouledogues français en bonne santé

L'indice de tonicité vaso-vagal (VVTI) est un indicateur temporel de la variabilité de la fréquence cardiaque; il est décrit comme un outil de diagnostic et de pronostic chez les chiens atteints de maladie cardiaque et d'insuffisance cardiaque. Les races brachycéphales ont tendance à avoir un VVTI plus élevé que les races non brachycéphales.[11]
Le but de cette étude était de déterminer les valeurs de référence VVTI à partir d'une cohorte représentative de bouledogues français en bonne santé à l'aide d'un enregistrement ECG. Un deuxième objectif de cette étude était d'évaluer la faisabilité du dépistage du VVTI dans

Determinazione delle norme di riferimento per l'indice del tono vasovagale in una coorte di Bulldog francesi sani.

L'indice del tono vasovagale (VVTI), un indicatore di dominio del tempo della variabilità della frequenza cardiaca, è stato suggerito come strumento diagnostico e prognostico nei cani affetti da malattia cardiaca e insufficienza cardiaca. Le razze brachicefaliche tendono ad avere un VVTI più elevato rispetto alle razze non- brachicefaliche[11]. L'obiettivo di questo studio è di ottenere valori di riferimento VVTI standard da parte da una coorte rappresentativa di Bulldog francesi sani basati su una singola registrazione ECG. Il secondo obiettivo era quello di valutare la praticabilità di uno screening VVTI di routine, valutando la ripetibilità del VVTI tra cinque la pratique clinique quotidienne en examinant la répétabilité du VVTI sur cinq mesures consécutives. Afin de déterminer les facteurs de base susceptibles d'affecter le VVTI, les effets du stress, de l'activité et du caractère, ainsi que le sexe et le statut de castration ont été évalués. Sur les 73 bouledogues français examinés, 60 chiens ont été jugés en bonne santé sur la base de leurs antécédents médicaux, de leur examen médical, de leur tension artérielle et de leur électrocardiogramme et aucun examen échocardiographique n'a révélé de maladie cardiaque congénitale ou acquise. Le VVTI a été calculé sur la variance de 20 intervalles R-R et sur la fréquence cardiaque extrapolée (HR) correspondante. De plus, un maximum de cinq valeurs VVTI consécutives ont été calculées pour chaque chien sur la base de 100 intervalles R-R consécutifs.

Les valeurs de VVTI allaient de 5,66 à 11,3 avec une moyenne (écart type [SD] de 8,82 (1,43). La fréquence cardiaque correspondante était comprise entre 78 et 173 battements par minute avec une moyenne (SD) de 120 (23) battements par minute. Pour l'interprétation clinique de VVTI, la corrélation négative entre VVTI et HR (R = 0,689; R2 ajusté = 0,466) doit être prise en compte. La répétabilité a été vérifiée sur cinq séries consécutives de 20 pulsations (variabilité intra-sujet moyenne de 6,1%). Le stress a affecté de manière significative le VVTI et la HR ($p < 0{,}001$). La gamme de VVTI identifiée dans cette étude peut être utilisée comme référence pour évaluer la variabilité de la fréquence cardiaque chez les races brachycéphales lors d'examens de santé de routine. Les écarts par rapport aux valeurs de référence permettent au clinicien d'ajuster le suivi.

Mots-clés: Brachycéphalie, chien, variabilité de la fréquence cardiaque, VVTI

misurazioni successive. Alfine di determinare i fattori chiave che potrebbero influenzare il VVTI, si sono valutati l'impatto dello stress, l'attività e il carattere, nonché il sesso e lo stato di castrazione/sterilizzazione. Su 73 Bulldog francesi controllati, 60 sono stati considerati sani sulla base di anamnesi, esame fisico, pressione sanguigna e ECG, e nessuna prova di malattia cardiaca congenita o acquisita dall'esame ecocardiografico. Il VVTI è stato calcolato sulla base della varianza di 20 intervalli R-R e l'HR corrispondente è stato estrapolato a un minuto dagli stessi 20 intervalli R-R. Inoltre, sono stati calcolati un massimo di cinque valori VVTI consecutivi sulla base di 100 intervalli R-R successivi registrati su ciascun cane. I valori VVTI variavano da 5,66 a 11,3 con una media (standard deviazione [SD]) di 8,82 (1,43); l'HR corrispondente variava da 78 a 173 bpm con una media (SD) di 120 (23) bpm. È importante notare che i valori di VVTI e HR erano correlati negativamente (R = 0,689; corretto R2 = 0.466), questo fattore deve venir preso in considerazione per l'interpretazione clinica del VVTI. La ripetibilità tra cinque serie successive di 20 battiti cardiaci è stata dimostrata (variabilità media intra-individuale del 6,1%). Lo stress ha influenzato significativamente il VVTI e l'HR ($p < 0{,}001$). L'intervallo VVTI stabilito in questo studio può essere utilizzato come riferimento per valutare l'HRV presumibile nei cani brachicefalici sani durante i controlli sanitari di routine. Deviazioni dal riferimento possono consentire al veterinario di adattare il programma e gli obiettivi delle successive indagini di follow-up.

Parole chiave: Brachicefalico, Cane, Variabilità della frequenza cardiaca, VVTI

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