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Effects of Behavior Responses on the Vasovagal Tonus Index in Healthy Dogs

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Original Article

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Abstract

The vasovagal tonus index (VVTI) is a time-domain analysis method of heart rate variability acquired over a short period. It is a useful measurement for evaluating severity and prognosis heart failure in dogs. Behavior responses can be used to evaluate stress in each dog individually. Stress during clinical examination may interfere with the VVTI since it influences the sympathetic nervous system. The aim of this study was to investigate the effect of behavior responses during clinical examination on the VVTI. Data set obtained from physical examination, systolic blood pressure measurement, electrocardiography, VVTI calculation, and video recording were collected from 50 healthy dogs. Behavior scores were analyzed from video recording and dogs were classified into three groups; group 1 (passive), group 2 (quite active), and group 3 (highly active). The results showed that the VVTI was not different between the three groups (p=0.77). Medians and interquartiles of the VVTI in group 1, 2, and 3 were 8.45 (6.86-9.05), 7.65 (6.82-8.94), and 7.26 (5.80-8.90) respectively. There was a negative correlation between VVTI and heart rate (Pearson's r= -0.68, p<0.001). Therefore, the effect of behavior responses during clinical examination did not affect the VVTI measurement in healthy dogs.

Keywords: behavior,dog, heart rate variability, stress, sympathetic,vasovagal tonus index

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บทคัดย่อ

ผลของพฤติกรรมต่อความแปรปรวนของอัตราการเต้นหัวใจในสุนัขปกติ

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Vasovagal tonus index (VVTI) เป็นวิธีการวัดความแปรปรวนของอัตราการเต้นหัวใจแบบขึ้นอยู่กับเวลาที่สามารถทำได้ในช่วงเวลาสั้นๆซึ่งเป็นตัวชี้วัดที่มีประโยชน์ในการประเมินความรุนแรงและการพยากรณ์โรคหัวใจล้มเหลวในสุนัข การตอบสนองทางพฤติกรรมสามารถนำมาใช้ในการประเมินความเครียดในสุนัขได้ จุดประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาพฤติกรรมที่แสดงออกในระหว่างการตรวจร่างกายทางคลินิก โดยบันทึกวิดีโอตลอดระยะเวลาที่สุนัขอยู่ในห้องตรวจ เก็บข้อมูลเฉพาะตัวของสุนัขและข้อมูลจากการตรวจร่างกาย วัดคลื่นไฟฟ้าหัวใจและความดันเลือดและคำนวณค่า VVTI จากสุนัขสุขภาพดี 50 ตัว โดยแบ่งสุนัขออกเป็น 3 กลุ่ม ตามคะแนนพฤติกรรมที่ได้วิเคราะห์จากวิดีโอ โดยกลุ่มที่ 1 เป็นสุนัขที่เงียบ (passive) กลุ่มที่ 2 เป็นสุนัขที่ค่อนข้างกระตือรือร้นและว่องไว (quite active) และกลุ่มที่ 3 เป็นสุนัขกระตือรือร้นและว่องไวมาก (highly active) ผลการศึกษาพบว่า VVTI ทั้งสามกลุ่มไม่มีความแตกต่างกันทางสถิติ (p=0.77) ค่ามัธยฐานและค่า 25-75 เปอร์เซ็นไทล์ของ VVTI ในกลุ่ม 1 กลุ่ม 2 และกลุ่ม 3 เป็นตามลำดับดังนี้ 8.45 (6.86-9.05), 7.65 (6.82-8.94) และ7.26 (5.80-8.90) พบความสัมพันธ์เชิงผกผันระหว่าง VVTI และอัตราการเต้นหัวใจ (Pearson's r= -0.68, p<0.001) ดังนั้นผลของการตอบสนองต่อพฤติกรรมในระหว่างการตรวจทางคลินิกไม่ได้มีผลกระทบต่อการวัด VVTI จากผลของการศึกษาในครั้งนี้แสดงให้เห็นว่าความเครียดที่เกิดขึ้นในขณะทำการตรวจทางคลินิกไม่ได้มีผลต่อค่า VVTI และสามารถนำค่า VVTI ไปใช้เป็นเครื่องมือประกอบการวินิจฉัยและพยากรณ์โรคหัวใจในสุนัขได้

คำสำคัญ: พฤติกรรมสุนัข ความแปรปรวนของอัตราการเต้นหัวใจ ความเครียด ซิมพาเทติก

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Introduction

Heart rate variability (HRV) describes the pattern of variation in heart rate between consecutive heart beats. HRV is an outcome of the relative equilibriums of the autonomic input to the sinoatrial node (Electrophysiology, 1996). Therefore, evaluation of HRV is a practical noninvasive procedure in an indirect measurement of parasympathetic activity (Häggström et al., 1996; Doxey and Boswood, 2004). HRV manifests the total variation in instantaneous heart rate (van RavenswaaijArts et al., 1993; Stein et al., 1994). There are many techniques for assessing HRV, which are categorized into two major categories; time-domain and frequency-domain. Time-domain analysis describes the changes in heart rate over time intervals between successive normal cardiac cycles, including calculation of RR interval average (mean, variances, and root mean square values). Frequency-domain analysis includes calculation of RR interval frequency transformations, for example power spectral analysis. In humans, time- and frequency-domain measures of HRV are related, each frequency-domain variable correlating with a time-domain variable (Stein et al., 1994). The standard deviation of normal sinus RR intervals (SDNN) is probably the simplest time-domain index of HRV. Decreased SDNN reflects sympathetic predominance or decreased parasympathetic tone to the sinoatrial node (Fujii and Wakao, 2003). SDNN has been considered as a useful predictor of development of atrial fibrillation, mortality, and progression to valve surgery in human patients with chronic severe mitral regurgitation (Stein et al., 1993). The vasovagal tonus index (VVTI) is a time-domain analysis of HRV calculated on the natural logarithm of the variance from R-R interval measured by twenty beat-to-beat RR intervals in the electrocardiograph (Häggström et al., 1996). It is widely used for sympathovagal balance studies as a diagnostic and prognostic tool for heart failure in dogs (Häggström et al., 1996; Doxey and Boswood, 2004; Pereira et al., 2008). Häggström et al. (1996) reported that the VVTI was related to severity of myxomatous mitral valve degeneration in the Cavalier King Charles Spaniels. Indeed, the VVTI decreased in dogs with congestive heart failure (Häggström et al., 1996; Doxey and Boswood, 2004). Moreover, it was suggested to be a useful prognostic marker in the canine dilated cardiomyopathy (Pereira et al., 2008).

The sympathovagal system regulates heart rate and rhythm determined by the intrinsic rate tone of the sinoatrial node varying according to a number of physiological factors, including exercise, stress, respiration and blood pressure regulation, the renin-angiotensin-aldosterone system and circadian rhythms (Calvert, 1998). Sympathetic stimulation accelerates heart rate, whereas parasympathetic (vagal) tone decreases it. In most physiological conditions, the activation of either sympathetic or vagal outflow is accompanied by the inhibition of the other, so that there is a pivotal balance between them (Malliani, 1999). Each heart beat is a discrete event. The length of cardiac cycle can be measured from the R-R wave intervals on the electrocardiogram (ECG). The variations in sympathetic and parasympathetic tone lead to fluctuations in the duration of cardiac cycle from beat to beat (Little and Julu, 1995). At rest, parasympathetic tone predominates in humans and dogs (Elsner et al., 1990; Little et al., 1999). Several studies showed that the VVTI is a useful method for evaluating the severity and predicting the prognosis of heart diseases in dogs (Häggström et al., 1996; Doxey and Boswood, 2004; Pereira et al., 2008). Little documented information reports how dogs cope during physical examination, or when admitted to veterinary care. Furthermore, little information shows the relationship between behaviors and the physiological changes. It is not only the emotional arousal, but also the physiological activities that reckon stress experienced. Moreover, different coping strategies may relate to different neurohormonal responses (Clark et al., 1997). As behavior signs can be used to evaluate stress in an individual, we are, therefore, interested that dogs differ when undergo procedures in consult room. To date, there is no evidence reporting the effect of behavior of the dog during physical examination on the sympathovagal system. Moreover, the changes that the VVTI undergoes in healthy dogs during the physical examination have not been described well. Therefore, the aim of this study was to examine the effect of behavior responses during physical examination on VVTI in healthy dogs.

Materials and Methods

Animals: The protocol used in this study was approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC). Client-owned dogs were enrolled into this prospective study at Prasu-Arthorn Animal Hospital, the Faculty of Veterinary Science, Mahidol University between March 2010 to May 2010. Fifty healthy dogs with age more than one year old were recruited into the present study. All dogs were considered healthy based on the database from history taking and physical examination.

Data collection: All procedures were performed in the same manner by the same two investigators in the same consult room and were recorded by a video recorder. Video recording started immediately when the owner and the dog entered the consult room. The owners were with their dogs throughout the examination. They were asked to free their dogs in the consult room for the first 3 minutes. The dogs were then subjected to a complete physical examination, including cardiac auscultation, heart rate and blood pressure measurement, and electrocardiography (ECG). Dogs were excluded from the study if they had evidence of any debilitating disorders.

Blood Pressure Measurement*:* The systolic arterial blood pressure was measured by Doppler flow detection system (model 811-B Doppler ultrasonic flow detector, Parks Medical Electronic Sales, Inc. Eastern Ave., Las Vegas, Nevada, USA) following a published protocol (Stepien and Rapoport, 1999). Briefly, an inflatable pressure cuff was wrapped around the antebrachium proximal to the carpal joint. The cuff with a width closest to 40% of the circumference of the antebrachium was selected. Dogs were allowed to adopt a comfortable position (usually right lateral recumbency) and the cuff was connected to a sphygmomanometer. The flow probe was positioned proximal to the palmar metacarpal pad at the level of the superficial palmar arterial arch and ultrasonic coupling gel was used. The position of the probe was adjusted until a clear signal was obtained. The cuff was inflated to 20-30 mmHg above the point at which the Doppler signal became inaudible. The cuff was slowly deflated and the pressure at which a signal could again be detected was recorded. This process was repeated until consistent consecutive readings were obtained. The first measurement was disregarded. Five systolic blood pressure readings were recorded and the arithmetic mean of these measurements was used for subsequent analysis.

Electrocardiography: Electrocardiograms recorded using a Cardisuny D300 (Fukuda ME, Koguo, Co Ltd, Japan) and a standard six-lead recording system (limb leads I, II, III, aVR, aVL and aVF). During the ECG recording, dogs were placed in right lateral recumbency with all four limbs perpendicular to the long axis of the body and slightly separate. Electrodes were attached to the skin on each of the four limbs using crocodile clips. Alcohol was used to improve the electrical contact. The ECG was recorded for approximately 10 sec for the six-lead ECG. A further 1 min of recording from lead II was obtained to assess cardiac rhythm. All measurements were derived from the lead II recording. Heart rate was measured by counting number of beats of 1 min recording. A paper speed of 25 mm/sec was used in every examination.

Calculation of Heart Rate Variability: The R-R wave intervals were measured for 20 consecutive beats from the first 20 beats of 1 min ECG recorded with the paper speed set at 25 mm/sec. Only good quality trace and continuous run of sinus rhythm for at least 20 R-R intervals were used. The variance (standard deviation of the R-R interval)^2, $(SD_{RR})^2$, for this

Figure 1 The R-R wave intervals in milliseconds (msec) were measured for 20 consecutive beats from the first 20 beats of 1 minute ECG recording with the paper speed set at 25 mm/second. The variance (standard deviation of the R-R interval)^2, $(SD_{RR})^2$, was calculated for each recording. The vasovagal tonus index was then calculated from the formula: $\ln(SD_{RR})^2$.

interval in millisecond was calculated for each recording (Fig 1). Premature beats were excluded and, in the case of ventricular premature beats, the following compensatory beat was also excluded (Stein et al., 1994; Calvert, 1998). The measurement of heart rate variability, the vasovagal tonus index (VVTI; a time-domain indicator), was calculated from the formula: VVTI = $\ln(SD_{RR})^2$ (Häggström et al., 1996; Doxey and Boswood, 2004; Pereira et al., 2008).

Analysis of behavior data: The video recording of the dogs' behavior were analyzed by 3 observers, started when the dogs were presented in the consult room to the end of all procedures (12-15 min in duration). Behavior was scored using a descriptive scale of 1-3 (Table 1). Each dog was scored independently by the three observers. Dogs were classified according to the median of the behavior scores obtained from the three observers. They were divided into three groups, group 1 (passive), group 2 (quite active), and group 3 (highly active). Agreement between observers was assessed (see in the statistical analyses).

Statistical Analyses: Computerized statistical software (SPSS 18.0 for Windows, Chicago, IL, USA) was used for all analyses. Each of the variables was assessed for significant difference between groups. All continuous variables were compared between groups by a Kruskal-Wallis test and a Mann-Whitney U test was used for post-hoc analysis. Chi-square test was used to compare categorical variable: sex. Pearson's correlation coefficient was employed to assess the relationship between the independent variables obtained from signalment and physical examination and dependent variables: VVTI and HR-PE. Wilcoxon signed-rank test was used to compare HR-PE and HR-ECG. Pairwise evaluation for agreement between observers on the categories of behavior scores was taken with the weighted kappa. Probabilities <0.05 were considered statistically significant. All analyses were two-tailed. Results were reported as median values with interquartile ranges.

Results

There were 50 dogs recruited to this study, 5 dogs were considered to be in group 1, 30 dogs were considered to be in group 2, and 15 dogs were considered to be in group 3. Five dogs in group 1 consisted of 4 males and one female. Breeds included Golden Retriever, Labrador Retriever, mixed breed, St. Bernard, and Thai Ridgeback. Thirty dogs in group 2 consisted of 20 males and 10 females. Breeds included 8 mixed breeds, 6 Shi Tzus, 4 Golden Retrievers, 3 Miniature Pinchers, 2 each of English Cocker Spaniels and Poodles, and one each of Beagle, Chihuahua, Siberian Husky, Spritz, and Thai Ridgeback. Fifteen dogs in group 3 consisted of 9 males and 6 females. Breeds included 3 each of mixed breeds and Shih Tzus, 2 each of Miniature Pinchers and

Table 1 Description of categories for behavior scores. Modified from Beerda et al. (1997).

Category (group)	Description
Passive (1)	Resting, watching surroundings but not seeking interaction, no attempts to flee, nor barking or howling
Quite active (2)	Postural changes (standing, sitting, walking, jumping, lying down), digging, scratching or biting the walls or equipments in the consult room, panting (at least 10 sec for each bout), barking or howling, oral behaviors (snout licking and smacking), yawning, tongue rolling, paw lifting, stretching, circling, body shaking, but such activities occurred less consistently and were accompanied by longer pauses than those seen in highly active
Highly active (3)	These activities last for at least 3 consecutive minutes: postural changes (standing, sitting, walking, jumping, lying down), digging, scratching or biting the walls or equipments in the consult room, panting (at least 10 sec for each bout), barking or howling, oral behaviors (snout licking and smacking), yawning, tongue rolling, paw lifting, stretching, circling, and body shaking

Poodles, and one each of Bangkaew, Chihuahua, Dachshund, Jack Russel Terrier, and Pomeranian. Chief complaints of these dogs were annual vaccination (n=26), heartworm prevention (n=19), annual vaccination and heartworm prevention (n=2), physical examination prior to sterilization (n=2), and physical examination for health certification to abroad (n = 1).

Clinical characteristics of passive (group 1), quite active (group 2), and highly active (group 3) are compared in Table 2. Body weight of dogs in group 1, group 2, and group 3 were significantly different (p=0.02). The body weight of dogs in group 1 was significantly heavier than dogs in group 2 (p=0.024), and group 3 (p=0.013). Heart rate measured from physical examination of dogs in group 1, group 2, and group 3 were significantly different (p=0.04). Heart rate measured from physical examination of dogs in group 1 was lower than in group 3 (p=0.027). There was no difference in age, heart rate measured from electrocardiography, systolic blood pressure and vasovagal tonus index between any of the groups (p=0.15, 0.17, 0.68, and 0.77, respectively).

There was a significant negative correlation between VVTI and HR-ECG (Pearson's r=-0.68, p<0.001), and HR-PE (Pearson's r=-0.51, p<0.001) (Table 3). There was a negative significant correlation between HR-PE and body weight (Pearson's r=-0.40, p=0.004), and age (Pearson's r=-0.28, p=0.048) (Table 4). The HR-PE was significantly correlated with the HR-ECG (Pearson's r=0.80, p<0.001) (Table 4). The HR-PE in 50 dogs was significantly higher than the HR-ECG (p=0.033). The median of HR-PE was 125 bpm (interquartile 110-150 bpm) and the median of HR-ECG was 117 bpm (interquartile 96-139 bpm). The HR-PE in group 1 and group 2 was not different from the HR-ECG (p=0.69 and p=0.28, respectively). The HR-PE in group 3 was significantly higher than the HR-ECG (p=0.031). During the approximately 10-min interval of the heart rate measurement by physical examination and electrocardiography, the heart rate decreased in 30 dogs, three of these were passive, sixteen intermediate, and eleven high individuals. In twelve dogs, the heart rate decreased over 10 beats per min, eight of these dogs were in the highly active group.

Agreement between observers for behavior scores was moderate to good. The weighted kappa's were 0.60, 0.54, and 0.46 for observers 1 VS 2, 1 VS 3, and 2 VS 3, respectively.

Table 2 Comparisons of dogs in group 1 (passive), group 2 (quite active), and group 3 (highly active) Results are in frequencies or medians (interquartile range).

Variable	Group 1 (n=5)	Group 2 (n=30)	Group 3 (n=15)	*P*
Age (years)	5.67 (2.54-10.71)	4.96 (2.98-7.21)	3.5 (2.25-5.08)	0.15
Male/Female (%)	4/1 (80/20%)	20/10 (67/33%)	9/6(60/40%)	0.68
Body weight (kg)	28.4 (15.1-41.2)[a,b]	9.4 (5.15-20.1)[a]	7.0 (4.2-17.0)[b]	0.02*
Heart rate - PE (bpm)	107 (82-122)[c]	125 (100-140)	150 (115-150)[c]	0.04*
Heart rate - ECG (bpm)	97 (90-112)	119 (94-135)	120 (104-149)	0.17
SBP (mmHg)	125 (104-142)	122 (108-137)	126 (118-140)	0.68
VVTI	8.45 (6.86-9.05)	7.65 (6.82-8.94)	7.26 (5.80-8.90)	0.77

*Significant difference between group 1, group 2, and group 3.
[a]Significant difference of the body weight between group 1 and group 2 (p=0.024).
[b]Significant difference of the body weight between group 1 and group 3 (p=0.013).
[c]Significant difference of heart rate measured from physical examination between group 1 and group 3 (p=0.027).
Heart rate – PE, heart rate measured from physical examination; Heart rate – ECG, heart rate measure from the electrocardiography; SBP, systolic blood pressure; VVTI, vasovagal tonus index.

Table 3 Pearson's Coefficient correlation between the dependent variable VVTI and the independent variables: age, body weight, heart rates measured from physical examination (HR-PE), heart rate measured from the ECG (HR-ECG), and systolic blood pressure.

	VVTI (Pearson's *r*)	*P*
Age (year)	-0.03	0.84
Body weight (kg)	0.08	0.59
HR-PE (bpm)	-0.51	< 0.001***
HR-ECG (bpm)	-0.68	< 0.001***
Systolic blood pressure (mmHg)	-0.05	0.71

Table 4 Pearson's Coefficient correlation between the dependent variable heart rates measured from physical examination (HR-PE) and the independent variables: age, body weight, heart rate measured from the ECG (HR-ECG), VVTI, and systolic blood pressure.

	HR-PE (Pearson's *r*)	*P*
Age (year)	-0.28	0.048*
Body weight (kg)	-0.40	0.004**
HR-ECG (bpm)	0.80	< 0.001***
VVTI	-0.51	< 0.001***
Systolic blood pressure (mmHg)	0.11	0.43

Discussion

This study showed that vasovagal tonus index (VVTI), heart rate measured from electrocardiography, and systolic blood pressure measured during clinical examination were not related to the behavioral response of the dogs. Although the investigators and environment were invariabile, a variety of behavioral response were observed in dogs subjected to the same manner of clinical examination. This behavioral response includes postural changes, digging, scratching or biting, panting, barking or howling, snout licking and smacking, yawning, tongue rolling, salivation, paw lifting, and body shaking. Indeed, these behavior responses were similar to the previous study in dogs experiencing acute stress (Beerda et al., 1998). These suggested that stress during physical examination did not affect the VVTI or systolic pressure in these dogs. In addition, the VVTI obtained from the dogs of each group was superior to the cut-off values of the VVTI in dogs with congestive heart failure in previous studies; less than 7.0 (Häggström et al., 1996), less than 6.75 (Doxey and Boswood, 2004), or less than 6.82 (Moonarmart, 2008). The VVTI had not different between normal dogs and dogs with asymptomatic myxomatous mitral valve disease (Häggström et al., 1996). Indeed, median of the VVTI in dogs with asymptomatic dilated cardiomyopathy (class 1 of the International Small Animal Cardiac Health Council, ISACHC) in the previous study was 7.7 (Pereira, et al., 2008) which was similar to values measured from healthy dogs in our study. This suggested that the VVTI in normal healthy dogs and dogs with asymptomatic heart disease was not clearly discriminated. This may indicate that the vagal tone in dogs with asymptomatic heart disease is well compensated. Moreover, this present study showed that the VVTI had significantly negative correlation with heart rate. This relationship was to be expected, as factors that increase heart rate also tend to decrease the variations in heart rate. This finding was in agreement with previous studies (Häggström et al., 1996; Doxey and Boswood, 2004; Moonarmart, 2008). Indeed, heart rate is influenced by changes of autonomic nervous system. It is characterized by an increase in sympathetic activity and a decrease in parasympathetic tone (Malliani, 1999).

It is of interest to note that heart rate measured from physical examination was higher than that from electrocardiography in the highly active dogs. This was in concert with the evidence of no difference in heart rate measured from electrocardiography among passive, quite active, or highly active dogs. These suggested that heart rate was increased by a high level of physical activity and decreased by a lower activity. According to the order of procedures in this study, the dogs were allowed to be free in the consult room for the first 3 min, and the heart rate measured from physical examination was then performed within 5 min. However, the heart rate measured from electrocardiography was performed when the dogs were handled to restrict their physical activity following physical examination and blood pressure measurement, respectively. This was similar to the evidence that cardiac responses depend on motor activity (Coote, 1975).

The results in the present study also showed that there was a weak negative correlation between heart rate and body weight, also a very weak negative correlation between heart rate and age. It was also shown that the small dogs seem to be more active when compared with larger dogs. These findings were similar to previous epidemiological studies, suggesting that heart rate in adult healthy dogs depends on breed type, body size, and age (Bodey and Michell, 1996; Michell, 1999). It is possible that heart rate and body weight may be linked to temperament and sympathetic influences during a presentation at a veterinary hospital. However, Lamb et al. (2010) reported that there was no correlation between body weight and heart rate in dogs that returned to their normal environment straight away followed by 24-hour Holter recording. (Lamb et al., 2010). It is important to note that the relationship between heart rate and body weight in Lamb et al. (2010) study was significant. Therefore, a larger number of dogs in a variety of sizes may be required for further investigation.

Weight kappa was used to compare the inter-observer variability of the behavior scores. The strength of agreement was simplified on the basis of the kappa values modified by Landis and Koch. Kappa values of 0.81 to 1.0 are considered excellent agreement, values of 0.61 to 0.80 are considered good agreement, values of 0.41 to 0.60 are considered moderate agreement, values of 0.21 to 0.40 are considered fair agreement, and values of less than 0.21 are considered poor agreement (Landis and Koch, 1977). In the present study, we found that inter-observer agreement was moderate. Some variability existed between observers despite the use of the description of category for behavior scores. Disagreement in interpretation was involved a slight variation in the rating of the behavior scores. A possible reason for this could be the wide-range degree of behavior response of each dog or different breeds.

In conclusion, our study showed that the effect of behavior responses during clinical examination did not affect the sympathovagal system in healthy dogs. Extrapolation from the finding in healthy dogs to dogs with heart failure; assuming that behavior responses of dogs with heart failure presented at veterinary practice is not influencing the sympathovagal system. Therefore, stress during clinical examination and procedures does not interfere in the VVTI measurement in dogs with heart disease. However, gentle handling of the dog during clinical examination should be performed to decrease stress and provide best animal welfare.

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