






## Evaluation of vasovagal tonus index and electrocardiographic parameters in horses using a new modified base apex lead method

Theerapong Pontaema<sup>1</sup> , Pongphol Pongthaisong<sup>1,2</sup> , Wootichai Kenchaiwong<sup>1,2,3</sup> ,  
Chayanon Chompoosan<sup>1</sup> , and Wichaporn Lerdweeraphon<sup>1,2</sup> 

1. Applied Animal Physiology Research Unit, Faculty of Veterinary Science, Maharakham University, Maharakham, 44000, Thailand; 2. Small Ruminant Research Unit, Faculty of Veterinary Science, Maharakham University, Maharakham, 44000, Thailand; 3. Network Center for Animal Breeding and Omics Research, Khon Kaen University, Khon Kaen, 40002, Thailand.

**Corresponding author:** Wichaporn Lerdweeraphon, e-mail: [wichaporn.l@msu.ac.th](mailto:wichaporn.l@msu.ac.th)

**Co-authors:** TP: [Theerapong.p@msu.ac.th](mailto:Theerapong.p@msu.ac.th), PP: [pongphol.p@msu.ac.th](mailto:pongphol.p@msu.ac.th), WK: [wootken@gmail.com](mailto:wootken@gmail.com),  
CC: [chayanon.c@msu.ac.th](mailto:chayanon.c@msu.ac.th)

**Received:** 13-03-2024, **Accepted:** 29-05-2024, **Published online:** 28-06-2024

**doi:** [www.doi.org/10.14202/vetworld.2024.1385-1390](http://www.doi.org/10.14202/vetworld.2024.1385-1390) **How to cite this article:** Pontaema T, Pongthaisong P, Kenchaiwong W, Chompoosan C, and Lerdweeraphon W (2024) Evaluation of vasovagal tonus index and electrocardiographic parameters in horses using a new modified base apex lead method, *Veterinary World*, 17(6): 1385–1390.

### Abstract

**Background and Aim:** Vasovagal tonus index (VVTI) serves as a straightforward assessment tool for autonomic function during both physiological and pathological conditions, including pregnancy, in horses. Obtaining VVTI through a modified base apex lead system could be a practical and comfortable solution. In this study, we assessed VVTI in horses with respect to training status and electrocardiographic measurements utilizing a novel modified base apex lead technique.

**Materials and Methods:** A total of 12 Thai native crossbred horses and 12 Arabian horses, all free of cardiac abnormalities, were enrolled in the study. Animals underwent electrocardiogram (ECG) and VVTI using both the base-apex lead method and its modified version. 25 mm/s and 10 mm/mV ECG recordings provided standard bipolar limb leads. The amplitudes and durations of P waves, QRS complexes, PR interval, QT interval, and T duration were assessed. The T wave's shape was examined. Each recording's R-R interval was utilized to assess heart rate. Twenty consecutive beats were used to compute the variability of heart rate (VVTI).

**Results:** The P wave amplitude was the only significant difference ( $p < 0.05$ ) between the base apex lead method and the modified base apex lead method, with no variations in heart rate, P duration, PR interval, T duration, and QRS duration and amplitude. Both methods showed mainly biphasic T wave patterns. The VVTI values of all horses did not differ significantly between the base apex and modified base apex methods. There was no significant difference in VVTI between Thai crossbred horses and Arabian horses in either method.

**Conclusion:** This study provided the first evidence that VVTI can be evaluated using the modified base apex lead system and may be useful for cardiovascular function monitoring in horses.

**Keywords:** electrocardiogram, evaluation, horses, vasovagal tonus index.

### Introduction

Due to the limitations of routine clinical electrocardiogram (ECG) recording, cardiac abnormalities can be challenging to diagnose [1]. Cardiac abnormalities can be difficult to investigate due to insufficient routine clinical diagnosis by ECG recording [1]. Assessing heart rate variability (HRV) in a clinical context aids the evaluation of autonomic nervous functions. It is valuable for diagnosing cardiac arrhythmias, tracking recovery, determining overtraining status, and evaluating sport horse performance [2–4]. Telemetric ECG and ambulatory Holter systems enable rapid and noninvasive HRV assessments. Telemetric

electrocardiography (Holter monitoring), with ECG configuration and HRV records, is used for monitoring arrhythmias during exercise [2, 5–7]. The equipment is limited to stable rest or low-intensity exercises such as endurance for accurate evaluation in horses due to constraints like following the horse's path or staying within a radio signal's range [8]. The equipment is most suitable for monitoring sustained arrhythmias such as atrial fibrillation and ventricular premature beats, which need observation within 24 h [1, 9–11]. Telemetric ECG is not affordable in Thailand. A 3 or 4 electrode resting digital ECG system has non-invasive and cost-effective abilities, along with adequate sensitivity for accurate heart rate rhythm identification, as described by Van Loon [12]. It can also evaluate HRV in short-term recording or vasovagal tonus index (VVTI) (time domain analysis of HRV) for the diagnosis of stress [13], pregnancy [14], and cardiac disease [15]. The relationship between training status and VVTI in horses is undefined.

In horses, an ECG is typically performed using the base-apex lead system, which places electrodes

Copyright: Pontaema, *et al.* Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

on the skin surface based on Einthoven's design. To obtain the largest complex on the electrode and facilitate interpretation, place the electrodes in line with the main depolarization direction, which is dorsal for ventricular activation and cranial for atrial activation [12]. This is due to the widespread Purkinje network extending throughout the ventricular myocardium, resulting in multiple points of depolarization across the myocardium, which is different from that observed in humans and small animals [12]. Optimal electrode positions for good quality ECG recordings have been extensively researched, decreasing movement artifacts and facilitating effortless interpretation [16–19]. The QRS complex amplitude and duration are unrelated to ventricular size. A four-electrode system with a modified base-apex recording could accurately diagnose heart rate and rhythm at rest. The electrodes should be placed along the main direction of depolarization, resulting in the largest complex, and will facilitate interpretation because the cardiac vector of the ventricular activation is dorsal, whereas the atrial activation is cranial with respect to the body surface [12]. The QRS complex and heart rate/VVTI measurement should be positioned vertically for optimal clinical observation and data acquisition.

Positioning electrodes vertically on the right side of a horse, with the positive at the 5<sup>th</sup> intercostal space and the negative at the right jugular furrow, could potentially improve signal quality and reduce movement artifacts. This new modified base apex lead system is suitable for obtaining VVTI from racing horses, providing routine monitoring of cardiovascular function and performance.

This study aimed to assess electrocardiographic parameters and VVTI between untrained (Thai native crossbred) and trained (Arabian) horses using a new modified base apex lead method.

## Materials and Methods

### Ethical approval

All procedures performed on animals in this study were approved by the Institutional Animal Ethics Committee, Maharakham University, Thailand (Approval number: IACUC-MSU-52/2023).

### Study period and location

The study was conducted from May to July 2023 at the Husbandry Section of the 2<sup>nd</sup> Livestock and Agriculture Division, Veterinary and Remount Department, Tha Phra Subdistrict, Mueang District, Khon Kaen Province, Thailand, Faro Farm, Mueang District, Roi Et Province, and SK Horse Stables, Mueang District, Sakon Nakhon Province, Thailand.

### Animals

A total of 24 healthy female horses, including Thai native crossbred horses ( $n = 12$ ) and Arabian horses ( $n = 12$ ) aged  $6.0 \pm 2.1$  years with a body weight of  $419.8 \pm 51.7$  kg, were used in this study. Animals without any cardiac problems, such as heart murmurs,

cardiac arrhythmias, or structural heart abnormalities, were included in the study. The criteria for cardiac problems were based on the results of auscultation and resting ECG recordings and echocardiography.

### ECG examination

ECG recordings were performed on animals using a 3-channel electrocardiograph (Edan Instruments, Inc., VE-300, China) at a paper speed of 25 mm/s and calibration of 10 mm equal to 1 mV. Before recording, the animals were restrained in a standing position without any chemical restraint for acclimation within 5 min. ECG recordings for all horses were made using the base-apex lead method and immediately followed by the modified method. Four electrodes were placed on unshaved skin with alligator clips for all standard bipolar limb leads (leads I, II, and III) and unipolar augmented limb leads (lead aVR, aVL, and aVF) in 1 min. The positions of the four electrodes in the two methods are shown in Table-1 and Figure-1. Alcohol was applied to improve electrical contact. The ECG was recorded for approximately 1 min for each lead system. The recording from lead I was used to evaluate heart rate (HR) and ECG parameter measurements, including P wave duration and amplitude, PR interval, QRS duration and amplitude, and QT interval. The T wave morphology was observed with positive and negative deflection and biphasic patterns. Heart rate was evaluated using the R-R interval. All analyses of ECG recordings were performed by the same researcher.

### Calculation of the HRV

All horses were evaluated for VVTI in this study. VVTI is a time-domain indicator of HRV analysis. VVTI measurement used short-term recordings and may be appropriate for resting horses. Good quality ECG traces and continuous running of sinus rhythm at the first 20 consecutive R-R intervals of 1 min in each ECG recording were selected for HRV measurement. HRV (VVTI) was obtained by calculating the variance standard deviation of the R-R interval<sup>2</sup> for this interval in milliseconds and the natural logarithm of the variance of the 20 measured R-R intervals, as described by the equation:  $VVTI = NL (\text{VAR} [R-R1 - R-R20])$ , where NL is the natural logarithm and VAR is the variance.

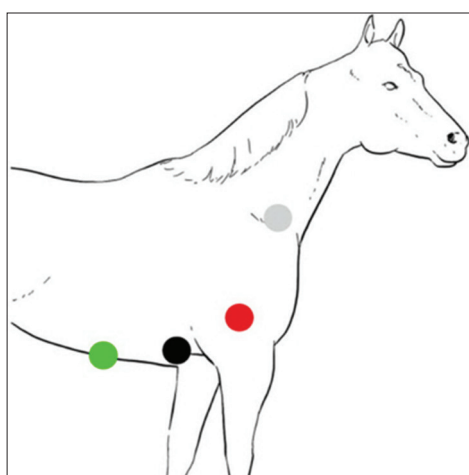
### Statistical analysis

All data were analyzed using an independent t-test with SAS software University Edition (SAS, Inc., Cary, NC, USA), and  $p < 0.05$  was considered statistically significant. The variation in ECG parameters between the two methods was examined to obtain the percentage of coefficient of variation (%CV). Descriptive statistics of the VVTI used a Kruskal–Wallis test.  $p < 0.05$  was considered statistically significant.

## Results

The ECG parameters are presented with their respective mean, standard deviation, and 95%

confidence interval (Table-2). ECG parameters were determined using the base apex lead and the modified base apex lead system, as depicted in Figures-2a and b. The P wave amplitude differed significantly between the two methods ( $p < 0.05$ ). The P wave's amplitude was lower in the modified base apex method compared to the base apex method. The other ECG parameters, including HR, P-wave duration, PR interval, QRS duration and amplitude, QT interval, and T-wave duration, showed no significant difference between the two methods. The T wave displayed a predominantly biphasic morphology in both the base apex and modified base apex methods. In Table-3, there was no significant difference in the coefficient of variation of the ECG parameters between the two



**Figure-1:** Electrode placement of the modified base apex lead method.

**Table-1:** Electrode placement.

| Electrode                      | Base apex lead method   | Modified base apex lead method   |
|--------------------------------|---|--|
| LA (black)                     | At 5 <sup>th</sup> intercostal space, just behind the point of the elbow of the left forelimb | At 5 <sup>th</sup> intercostal space, just behind the point of the elbow of the right forelimb |
| RA (white)                     | At the right jugular furrow or in front of right scapula spine                                | Same position in Base apex lead method   |
| LF (red)                       | On the loose skin at the left stifle in the region of the patella                             | On the skin at the triceps brachii of the right forelimb                                       |
| RF (green or ground electrode) | On the loose skin at the right stifle in the region of the patella                            | On the skin at the xiphoid process   |

LA=Left arm, RA=Right arm, LF=Left foot, RF=Right foot

**Table-2:** Comparison of the electrocardiographic parameters (mean  $\pm$  SD) between the base apex and the modified base apex method.

| ECG parameters        | Base apex lead method |                      | Modified base apex lead method |                      | p-value |
|-----------------------|-----------------------|----------------------|--------------------------------|----------------------|---------|
|                       | Mean $\pm$ SD         | 95% CI (lower-upper) | Mean $\pm$ SD                  | 95% CI (lower-upper) |         |
| HR (beats/min)        | 37.44 $\pm$ 7.57      | 37.44-40.64          | 36.03 $\pm$ 7.42               | 36.03-39.16          | 0.4978  |
| P wave duration (s)   | 0.13 $\pm$ 0.03       | 0.13-0.14            | 0.12 $\pm$ 0.04                | 0.12-0.14            | 0.5653  |
| P wave amplitude (mV) | 0.18 $\pm$ 0.07       | 0.18-0.20            | 0.13 $\pm$ 0.05                | 0.13-0.15            | 0.0064  |
| PR interval (s)       | 0.26 $\pm$ 0.06       | 0.26-0.29            | 0.27 $\pm$ 0.06                | 0.27-0.29            | 0.9625  |
| QRS duration (s)      | 0.12 $\pm$ 0.01       | 0.12-0.13            | 0.12 $\pm$ 0.02                | 0.12-0.13            | 0.8465  |
| QRS amplitude (mV)    | 1.43 $\pm$ 0.33       | 1.43-1.57            | 1.45 $\pm$ 0.33                | 1.45-1.59            | 0.7918  |
| QT interval (s)       | 0.47 $\pm$ 0.04       | 0.47-0.49            | 0.46 $\pm$ 0.04                | 0.46-0.48            | 0.3555  |
| T wave duration (s)   | 0.12 $\pm$ 0.04       | 0.12-0.14            | 0.12 $\pm$ 0.03                | 0.12-0.13            | 0.477   |

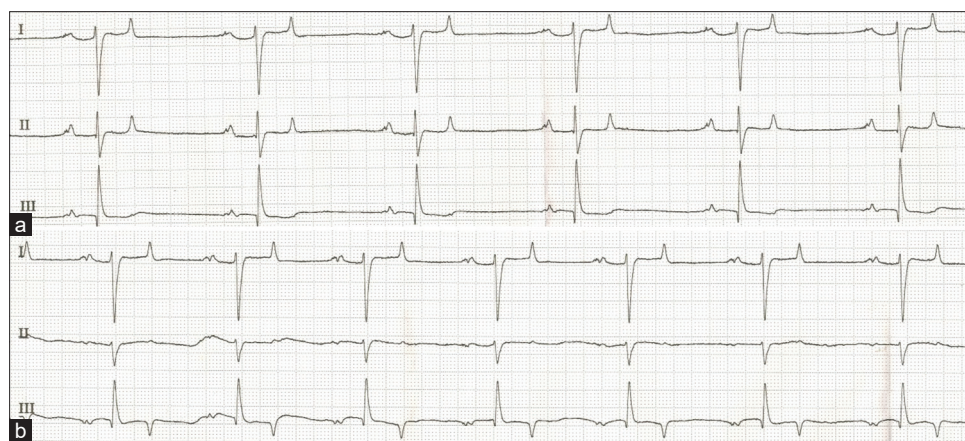
SD=Standard deviation, ECG=Electrocardiogram, HR=Heart rate, CI=Confidence interval

methods. The descriptive statistics of VVTI were equivalent between the base apex and modified base apex methods (Table-4). The VVTI in Thai crossbred and Arabian horses were comparable in both methods (Table-5).

## Discussion

Both lead methods yielded similar resting HRs for horses. The study found that the modified base apex lead HR and rhythm monitoring method was equivalent to the standard method in sport horses. The electrodes were positioned more vertically to ensure clear QRS amplitude and observe the main ventricular depolarization direction. The P wave amplitude was less in the modified base apex lead method than the base apex lead method, but the P wave duration remained unchanged. The atrial activation might have proceeded in a more cranial-caudal direction instead of the main ventricular activation's ventral-dorsal direction [12]. Atrial activity may not be optimally recorded using these electrode positions. The other ECG parameters of the modified base apex lead method, identical to previous reports [14, 16, 17], were all normal. Despite the trend of rising QRS duration variation in the modified base apex lead method, the duration proved insignificant for determining ventricular size and, thus, unnecessary for diagnosing equine cardiac hypertrophy. Instead of the standard base apex lead method, the modified base apex lead method can be used for heart rate and rhythm observation in resting ECG recording of horses.

VVTI, derived from a 20-beat time series analysis of HRV, is a simple method for evaluating



**Figure-2:** Example of electrocardiogram recording in lead I, II, and III for (a) the base apex lead method (b) and the modified base apex lead method from the same horse (paper speed = 25 mm/s, sensitivity = 10 mm/mV).

**Table-3:** The %CV of ECG parameters between two methods.

| ECG parameters        | %CV                   |                                | p-value of equality of variances |
|-----------------------|-----------------------|--------------------------------|----------------------------------|
|                       | Base apex lead method | Modified base apex lead method |                                  |
| HR (beats/min)        | 20.22                 | 20.60                          | 0.92                             |
| P wave duration (s)   | 23.37                 | 32.04                          | 0.25                             |
| P wave amplitude (mV) | 39.43                 | 38.36                          | 0.15                             |
| PR interval (s)       | 23.97                 | 21.89                          | 0.71                             |
| QRS duration (s)      | 9.27                  | 13.78                          | 0.07                             |
| QRS amplitude (mV)    | 22.86                 | 22.39                          | 0.99                             |
| QT interval (s)       | 9.23                  | 9.46                           | 0.99                             |
| T wave duration (s)   | 32.87                 | 26.62                          | 0.25                             |

ECG=Electrocardiogram, HR=Heart rate, %CV=Percentage coefficient of variation

**Table-4:** Descriptive statistics of VVTI in each method.

| Statistical measures       | Base apex lead method (n = 24) | Modified base apex lead method (n = 24) | Kruskal-Wallis test (p-value) |
|----------------------------|--------------------------------|---|-------------------------------|
| Basic statistical measures |                                |   |                               |
| Mean ± SD                  | 9.18 ± 1.25                    | 9.2070 ± 0.96                           |                               |
| Median                     | 8.83                           | 9.18                                    | 0.91                          |
| Standard error of mean     | 0.25                           | 0.19                                    |                               |
| Skewness                   | 0.14                           | 0.45                                    |                               |
| Coefficient variation      | 13.62                          | 10.46                                   |                               |
| Variability                |                                |   |                               |
| Variance                   | 1.56                           | 0.93                                    |                               |
| Range                      | 5.46                           | 4.16                                    |                               |
| Interquartile Range        | 1.55                           | 1.14                                    |                               |

VVTI=Vasovagal tonus index, SD=Standard deviation

**Table-5:** Descriptive statistics of VVTI in each method by breeds.

| Statistics               | Base apex lead method     |                  | Kruskal-Wallis test (p-value) | Modified base apex lead method |                  | Kruskal-Wallis test (p-value) |
|--------------------------|---------------------------|------------------|-------------------------------|--------------------------------|------------------|-------------------------------|
|                          | Thai cross breed (n = 12) | Arabian (n = 12) |                               | Thai cross breed (n = 12)      | Arabian (n = 12) |                               |
| Minimum                  | 7.8                       | 6.4              |                               | 7.4                            | 7.7              |                               |
| 25% Percentile           | 8.3                       | 8.2              |                               | 8.4                            | 8.5              |                               |
| Median                   | 9.1                       | 9.0              | 0.88                          | 9.1                            | 9.3              | 0.68                          |
| 75% Percentile           | 9.9                       | 10.4             |                               | 9.6                            | 10.0             |                               |
| Maximum                  | 11.8                      | 11.3             |                               | 11.6                           | 10.8             |                               |
| Coefficient of variation | 11.39%                    | 15.04%           |                               | 11.4                           | 8.90             |                               |

VVTI=Vasovagal tonus index

sympathetic and parasympathetic control balance. In resting horses, VVTI is an appropriate method for

assessing cardiac function [8, 14]. The modified base apex lead method and base apex lead method can

calculate VVTI values of 9.18 and 8.83, respectively. Placing electrodes vertically on the right side according to the modified base apex lead method can assess VVTI in resting horses. This technique could simplify electrode attachment and indicate when they detach from the skin.

High-intensity training and overtraining in sport horses alter their cardiovascular function through modifications in autonomic nervous regulation [3]. HRV can be utilized to assess cardiovascular responses to various training techniques in horses. Time-domain HRV analysis methods such as VVTI have not been compared between untrained and trained horses yet. Despite the use of two different analysis methods, VVTI remained unaltered for both Thai cross-breed and Arabian horses (VVTI values: 9.1 and 9.0 for base apex lead method, and 9.1 and 9.3 for modified base apex lead method). The data suggested that VVTI remained unchanged regardless of the horses' training status. Although VVTI uses only short-term recordings, these data are an indicator of vagal control of heart rate and may, therefore, be appropriate in the resting horse [8]. HRV analysis in the frequency domain should be performed during exercise because it is a sensitive method for assessing exercise response to cardiovascular function [20]. Thus, telemetric ambulatory ECG with portable recording and storage of ECG data is suitable for time or frequency domain analysis of HRV in long-term recordings in horses [21, 22].

### Conclusion

The new modified base-apex lead method, as shown in this study, is a dependable approach for diagnosing heart rate and rhythm in horses through VVTI analysis. Identifying the P wave configuration accurately is a limitation of this method.

### Authors' Contributions

WK: Designed the study and analyzed the data. CC, PP, TP, and WL: Recorded and analyzed the data. WL: Coordinated the study and wrote and revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

### Acknowledgments

This study was financially supported by Mahasarakham University, Thailand, 2023 (grant number: 6608012). The authors are thankful to the Husbandry Section of the 2<sup>nd</sup> Livestock and Agriculture Division, Veterinary and Remount Department, Tha Phra Subdistrict, Mueang District, Khon Kaen Province and Faro Farm, Mueang District, Roi Et Province, Thailand, for providing the horses and necessary facilities to conduct the study. In addition, the authors are very thankful to J.F. Advance Med Co., Ltd, Khlong Chaokhun Sing, Wang Thonglang, Bangkok, Thailand for ultrasound system support.

### Competing Interests

The authors declare that they have no competing interests.

### Publisher's Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

### References

1. Van Loon, G. (2013) ECG Survival Tips: How to Record them and how to Read them. Conference: BEVA Winter Clinical Meeting: Equine Cardiology Updates, London, UK.
2. Hammond, A., Sage, W., Hezzell, M., Smith, S., Franklin, S. and Allen, K. (2023) Heart rate variability during high-speed treadmill exercise and recovery in Thoroughbred racehorses presented for poor performance. *Equine Vet. J.*, 55(5): 727–737.
3. Kinnunen, S., Laukkanen, R., Haldi, J., Hanninen, O. and Atalay, M. (2006) Heart rate variability in trotters during different training periods. *Equine Vet. J. Suppl.*, 38(36): 214–217.
4. Szabo, C., Vizesi, Z. and Vincze, A. (2021) Heart rate and heart rate variability of amateur show jumping horses competing on different levels. *Animals (Basel)*, 11(3): 693.
5. Frick, L., Schwarzwald, C.C. and Mitchell, K.J. (2019) The use of heart rate variability analysis to detect arrhythmias in horses undergoing a standard treadmill exercise test. *J. Vet. Intern. Med.*, 33(1): 212–224.
6. McCrae, P., Spong, H., Golestani, N., Mahnam, A., Bashura, Y. and Pearson, W. (2023) Validation of an equine smart textile system for heart rate variability: A preliminary study. *Animals*, 13(3): 512.
7. Alberti, E., Stucchi, L., Lo Feudo, C.M., Stancari, G., Conturba, B., Ferrucci, F. and Zucca, E. (2021) Evaluation of cardiac arrhythmias before, during, and after treadmill exercise testing in poorly performing standardbred racehorses. *Animals (Basel)*, 11(8): 2413.
8. Marr, C.M. and Bowen, I.M. (2010) *Cardiology of the Horse*. 2<sup>nd</sup> ed. Saunders, Edinburgh, New York, p132.
9. Broux, B., De Clercq, D., Vera, L., Ven, S., Deprez, P., Van Loon, G. and Decloedt, A. (2018) Can heart rate variability parameters derived by a heart rate monitor differentiate between atrial fibrillation and sinus rhythm? *BMC Vet. Res.*, 14(1): 320.
10. Mitchell, K.J. and Schwarzwald, C.C. (2021) Heart rate variability analysis in horses for the diagnosis of arrhythmias. *Vet. J.*, 268: 105590.
11. Broux, B., De Clercq, D., Decloedt, A., Ven, S., Vera, L. and Van Steenkiste, G. (2017) Heart rate variability parameters in horses distinguish atrial fibrillation from sinus rhythm before and after successful electrical cardioversion. *Equine Vet. J.*, 49(6): 723–728.
12. Van Loon, G. (2013) Electrocardiographic Diagnosis: Are we Trying Hard Enough? Conference: Forum of the American College of Veterinary Internal Medicine. Seattle, US.
13. Von Borell, E., Langbein, J., Despres, G., Hansen, S., Leterrier, C. and Marchant-Forde, J. (2007) Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals. *Physiol. Behav.*, 92(3): 293–316.
14. Chompoosan, C., Pongthaisong, P., Kenchaiwong, W., Pontaema, T. and Lerdweeraphon, W. (2023) Effects of pregnancy on electrocardiographic, vasovagal tonus index, and echocardiographic variables in horses. *Vet. World*, 16(8): 1765–1771.
15. Hammond, L. (2006) Activation of the Autonomic Nervous

- System in Horses with Aortic Valve Disease. Royal Veterinary College, London, p32.
16. Kenchaiwong, W., Sangpo, P., Kusol, A., Pontaema, T. and Lerdweeraphon, W. (2022) The position of ground electrode affects electrocardiographic parameters in horses. *Vet. World*, 15(4): 1107–1112.
  17. Cherdchutham, W., Koomgun, K., Singtoniwet, S., Wongsutthawart, N., Nontakanun N., Wanmad, W. and Petchdee, S. (2020) Assessment of cardiac variables using a new electrocardiography lead system in horses. *Vet. World*, 13(6): 1229–1233.
  18. Ayala, I., Gutierrez-Panizo, C., Benedito, J.L., Prieto, F. and Montes, A. (2000) Morphology and amplitude values of the electrocardiogram of Spanish-bred horses of different ages in the Dubois leads system. *Vet. Res.*, 31(3): 347–354.
  19. Hesselkilde, E.M., Isaksen, J.L., Petersen, B.V., Carstensen, H., Jespersen, T., Pehrson, S., Kanters, J.K. and Buhl, R. (2021) A novel approach for obtaining 12-lead electrocardiograms in horses. *J. Vet. Intern. Med.*, 35(1): 521–531.
  20. Physick-Sheard, P.W., Marlin, D.J., Thornhill, R. and Schroter, R.C. (2000) Frequency domain analysis of heart rate variability in horses at rest and during exercise. *Equine Vet. J.*, 32(3): 253–262.
  21. Norman, S.E., Eager, R.A., Waran, N.K., Jeffery, L., Schroter, R.C. and Marlin, D.J. (2005) Recording of ECG signals on a portable MiniDisc recorder for time and frequency domain heart rate variability analysis. *Physiol. Behav.*, 83(5): 729–738.
  22. Eggenesperger, B.H. and Schwarzwald, C.C. (2017) Influence of 2<sup>nd</sup>-degree AV blocks, ECG recording length, and recording time on heart rate variability analyses in horses. *J. Vet. Cardiol.*, 19(2): 160–174.

\*\*\*\*\*