# Signs of myocardial damage on electrocardiogram correlate with elevated plasma cardiac troponin T in sport horses

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## Abstract

Myocardial dysfunction in horses does not only impacts their performance but can also be life threatening. However, other than the basic assessments of myocardial function e.g. electrocardiography and echocardiography, there are no other diagnostic assays that can be used to detect myocardial damage. This study was performed to determine the correlation between the use of serum cardiac troponin T (cTnT) and the occurrence of electrocardiological abnormalities and to evaluate the presence and extent of myocardial injury in sport horses. Seventeen Thoroughbred and thirteen Argentine Polo pony horses with an age range of 3 to 21 years were assessed for cTnT concentrations and their electrocardiograms (ECGs) were recorded using a base-apex lead in a resting stage. Resting heart rate (38.9±2.4) of horse is higher than other recent reports. There was no significant difference between genders for all ECG parameters. Plasma cTnT was higher in horses with ECG changes (CD group; 0.007±0.0009) vs normal adult horses (N1 group (3-5 years old); 0.003±0.0003), $p<0.05$. Our study indicates that the cTnT assay is a reliable method for diagnosis of myocardial damage in sport horse.

**Keywords:** Cardiac marker, base-apex lead, horses

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# การบ่งชี้ความเสียหายของกล้ามเนื้อหัวใจจากความสัมพันธ์ระหว่างคลื่นไฟฟ้าหัวใจและระดับที่เพิ่มขึ้นของโทรโปนิน ที ในพลาสมาในม้ากีฬา

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**บทคัดย่อ**

ความผิดปกติในการทำงานของกล้ามเนื้อหัวใจในม้า มักส่งผลกระทบต่อประสิทธิภาพในการทำงาน และสามารถเป็นอันตรายถึงแก่ชีวิตได้ อย่างไรก็ตาม นอกจากการประเมินการทำงานของหัวใจพื้นฐาน เช่น การตรวจด้วยคลื่นไฟฟ้าหัวใจ และคลื่นเสียงสะท้อนหัวใจ ยังไม่มีการตรวจวินิจฉัยอื่นๆ ที่สามารถใช้ตรวจสอบความเสียหายของกล้ามเนื้อหัวใจได้ การศึกษาในครั้งนี้ได้ดำเนินการเพื่อตรวจสอบความสัมพันธ์ระหว่างการใช้ระดับโทรโปนิน ที ในกระแสโลหิต และความผิดปกติของคลื่นไฟฟ้าหัวใจ เพื่อกำหนดขอบเขตและประเมินการเกิดการบาดเจ็บของกล้ามเนื้อหัวใจในม้ากีฬา โดยใช้ม้าพันธุ์ Thoroughbred 17 ตัว และพันธุ์อาร์เจนตินาโปโล 13 ตัว ที่มีช่วงอายุระหว่าง 3-21 ปี นำมาประเมินปริมาณโทรโปนิน ที ในกระแสโลหิต และทำการบันทึกคลื่นไฟฟ้าหัวใจในขณะที่ม้าพัก โดยใช้การบันทึกคลื่นไฟฟ้าหัวใจที่ตำแหน่งจากฐานไปยอดหัวใจ พบว่าอัตราการเต้นของหัวใจในม้าขณะพัก (38.9±2.4) มีค่าสูงกว่ารายงานอื่นๆ ที่ผ่านมา และสำหรับค่าตัวแปรอื่นๆ ของคลื่นไฟฟ้าหัวใจ พบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในม้าเพศผู้และเพศเมีย การใช้ชุดทดสอบโทรโปนินเชิงพาณิชย์ในการตรวจตัวอย่างเลือดม้าจำนวน 25 ตัวอย่าง คิดเป็น 93 เปอร์เซ็นต์ ให้ผลลบต่อการตรวจสอบระดับโทรโปนิน ที ในพลาสมา และพบว่าปริมาณโทรโปนิน ที ในพลาสมามีปริมาณสูงอย่างมีนัยสำคัญทางสถิติ ในกลุ่มที่พบว่ามีความผิดปกติของคลื่นไฟฟ้าหัวใจ (กลุ่ม CD; 0.007±0.0009) เมื่อเปรียบเทียบกับม้ากลุ่มปกติที่มีอายุระหว่าง 3-5 ปี (กลุ่ม N1; 0.003±0.0003) โดยมี ค่า p-value น้อยกว่า 0.05 การศึกษาในครั้งนี้ พบว่าการทดสอบการบาดเจ็บของกล้ามเนื้อหัวใจจากปริมาณโทรโปนิน ที ในพลาสมา เป็นวิธีการที่เชื่อถือได้ และสามารถใช้เพื่อวินิจฉัยการบาดเจ็บของกล้ามเนื้อหัวใจในม้ากีฬา

**คำสำคัญ**: ตัวบ่งชี้หัวใจ การบันทึกคลื่นไฟฟ้าที่ตำแหน่งจากฐานไปยอดหัวใจ ม้า

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## Introduction

Myocardial damage is a common cause of poor performance and difficult to diagnose in horses [1,2]. Thoroughbred and Argentine Polo Pony horses are the breed of choice for equestrian sports worldwide [3,4]. A good physical condition is necessary for equestrian sports horses, at which the cardiovascular fitness affects the ability to bear a hard physical activity [5]. For many years, the body surface electrocardiogram (ECG) has been used in human and veterinary medicine for the diagnosis and treatment of cardiac arrhythmias, e.g. electrolyte imbalances, respiratory, and heart diseases [5,6]. ECG is regarded as the precise technique to determine the cardio-respiratory dysfunction [7]. Besides diagnosis and treatment of myocardial disease using ECG, cardiac marker tests in particular cardiac troponin (cTn), has been approved as a potential diagnostic tool for minor myocardial injury. A plasma cTnT assay, components of the troponin complex of muscle cells, has been used to identify myocardial damage in many species [8,9]. Troponins are regulatory proteins that involved in contraction and relaxation process in striated muscle as well as in cardiac muscle. Troponins contained 3 different subunits: troponin C, troponin T and troponin I. Troponin C is found in both skeletal and cardiac muscle, while troponin I and T are only found in cardiac muscle. Evaluation of cardiac troponin I and T levels have been selected as gold standard biomarkers for myocardial injuries. In human Cardiac troponin (cTn) is released within 4-12 hours after myocardial damage and high plasma levels are maintained in circulation for up to 8 days [10]. Human plasma cTn has been used to diagnose acute myocarditis and predict the subsequence of cardiac morbidity and mortality [11-13]. However, information about the efficacy of this assay for detecting equine myocardial dysfunction is presently limited. The purpose of this study is to examine the correlation between electrophysiological abnormalities and cTnT levels in a group of sport horses and to determine whether the commercial cTnT assay has the potential to diagnose myocardial damage.

## Materials and Methods

### Animals

The study was conducted in Thoroughbred and Argentine Polo Pony horses, databases in each group as shown in Table 1. This study was approved by the Ethical Committee for Animal Experiments, Kasetsart University, Thailand. Clinical evaluation consisted of physical

examination, electrocardiography, complete blood cell count and measurement of plasma cTnT concentration.

**Table 1.** Horses database

| Parameters | N1 | N2 | CD |
|---|---|---|---|
| Age (Years) | 3-5 | 6-17 | 8-21 |
| Breed (N) | Thoroughbred (7) | Thoroughbred (7) | Thoroughbred (3) |
| | Argentine Polo Pony (2) | Argentine Polo Pony (7) | Argentine Polo Pony (4) |

**Electrocardiogram recording**

The Electrocardiogram (ECG) parameter was measured in 18 female and 12 male horses at resting stage. The age of horses ranged from 3 to 21 years. ECGs were recorded in a base-apex lead as described by Robertson [14]. A portable, single-channeled, battery-driven (Kenz ECG110) apparatus was used at a paper speed of 25 mm/s and gain of 0.5 cm/mV. Morphology and amplitude of P wave, QRS complexes, and T waves were calculated and analysed in the base-apex lead. ECGs were employed for heart rate and rhythm. P wave was classified as two peaks of the same polarity (bifid) and positive and negative deflection of the P wave (biphasic). Furthermore, T wave was divided into four groups: (i) positive monophasic, (ii) negative monophasic, (iii) positive biphasic, and (iv) negative biphasic, according to the previous report [5].

**Measurement of plasma cardiac troponin T**

Plasma cardiac troponin T (cTnT) was measured using Roche Cardiac Troponin-T Sensitive kit and quantitative measurement by electrochemiluminescence (ECL) immune assay. Blood samples were collected by venipuncture and placed in standard glass tubes containing lithium heparin. All blood samples were centrifuged (2000g for 20 min) before the plasma was separated. Plasma samples were immediately transferred to micro-tube and placed in a freezer (-80$^{\circ}$ C) and the samples were transferred to laboratory services within 6 hours of collection. All samples were obtained 1-2 minutes before the start of electrocardiographic evaluation.

### Classification of myocardial damage

The degrees of myocardial damage is classified by severity level as mild, moderate and severe based on the medical history, clinical signs and electrocardiogram findings. The commercial cardiac Troponin T test kit was used to confirm the diagnosis of myocardial injury.

### Statistical analysis

Data in this study are presented using mean ± standard error of the mean (SEM). The study was performed in three groups of subjects, control group included 23 healthy normal adult horses: N1 group (N1) age range between 3-5 years, N2 group (N2) age range between 6-17 years and CD group (CD) included 7 horses that the presence of any electrocardiogram changes in which age range between 8-21 years. Group data were analyzed using a one way ANOVA test, where $p<0.05$ was considered significant. Concentration of plasma cTnT below the lowest detectable limit of assay was considered as a value, which is less than the limit (0.0029 in a limit of <0.003 ng/ml) for statistical test. Paired t-test was used to compare the heart rate between normal horses and horses that the presence of any electrocardiogram changes. Box and whisker plots were used to represent the description data of correlation between electrocardiogram and the level of plasma cTnT.

## Results

Thirty horses were studied (12 males and 18 females). The age range was from 3-21 years (9.5±1.0). None of the horses in control groups (N1 and N2) showed any significant findings on physical examination or on electrocardiogram (ECG) recording performed before the start of the experiment. The ECG morphologies with standard waves: P, Q, R, S, and T, also ECG parameters such as PR interval, QT interval, QRS complex, and T wave duration are shown in Figure 1. The electrocardiographs of 23 horses were classified as normal whereas 7 horses had abnormal ECG characteristics such as supraventricular tachycardia, first degree heart block, ST elevation and atrial flutter. The mean plasma cTnT concentrations of N1 group and CD group were statistically different (mean ± SEM, 0.003 ± 0.0003 ng/mL (N1) and 0.007 ± 0.001 ng/mL (CD)). Plasma cTnT concentration tended to be higher in CD as compared to N1 and N2 ($p<0.05$ of an ANOVA test, Fig. 2).

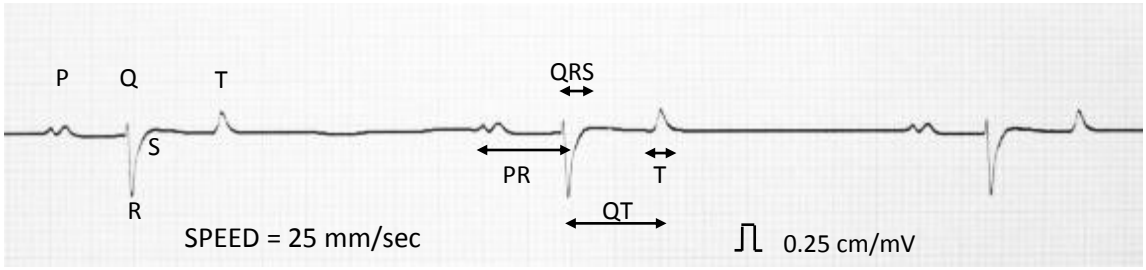


**Figure 1.** Base-apex lead ECG of normal horse. Note that ECGs are recorded at speed of 25 mm/sec and amplitude of 0.25 cm/mV

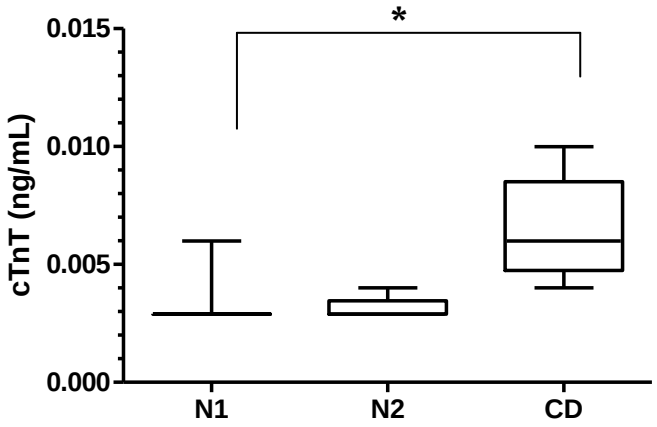


**Figure 2.** Box and whisker plots of plasma cTnT concentrations in 30 horses. The group was divided according to whether an abnormal or normal ECG was present. N1 group (N1) age range between 3-5 years, N2 group (N2) age range between 6-17 years and CD group (CD) age range between 8-21 years included 7 horses that the presence of any electrocardiogram changes. Distributions of plasma cTnT are represented as low, median and upper quartiles. Whiskers above and below box showed the $^{95th}$ and $^{5th}$ percentiles, respectively. Median plasma cTnT of horses with abnormal electrocardiogram (CD) were significantly higher than that of horses in normal groups (N1), $p < 0.05$ of an ANOVA test as indicated by (*).

Plasma cTnT concentration was significantly correlated with ECG parameters: P wave, QRS wave, T wave durations, and PR intervals ($r^{/2}$ = 0.690, 0.830, 0.755 and 0.708, respectively). Heart rate at rest has been considered as an indicator of cardiovascular fitness and the lower heart rate indicates the larger stroke volume [15]. Our study showed that heart

rate ranged from 25 to 60 beats per minute, with mean ± SEM of 38.9 ± 2.4. All horses showed no significant differences between the heart rates of the female horses (37.8 ± 1.7) and the male horses (40.5 ± 3.2). However, statistically significant differences between the heart rates of the horses in N1 group (35.3 ± 1.4) and the horses were found in CD group (47.1.0 ± 5.2).

The amplitude and duration of the electrocardiogram parameters are shown in Table 2 and 3. There appeared to be no statistically significant differences in ventricular depolarization between female and male horses. Almost all the horses (75%) showed a very high frequency of positive biphasic P wave whereas 4 horses (25%) were bifid (Table 3). Horses showed common biphasic P wave, which is different from previous studies that mainly showed the bifid P wave [5]. No statistically significant differences were observed in P wave parameter between female and male horses. Ventricular depolarization wave showed a morphological feature of three wave forms; Q, R, and S wave in all horses. The ECG base-apex lead of horses showed two forms of T waves; monophasic and biphasic. Forty-four percent of the T-wave patterns had monophasic pattern, while 56% of the T waves were biphasic. The durations of the electrocardiogram parameters in N1, N2 and CD groups are shown in Table 4.

**Table 2.** Duration (second) of Electrocardiogram parameters in sport horses (N=23)

| Parameters | N1 (9) (Mean±SEM) | N2 (14) (Mean±SEM) |
|---|---|---|
| P wave duration | 0.14±0.006 | 0.13±0.009 |
| QRS complex duration | 0.12±0.002 | 0.11±0.004 |
| T wave duration | 0.12±0.02 | 0.16±0.01 |
| PR interval | 0.35±0.01 | 0.33±0.02 |
| QT interval | 0.50±0.01 | 0.48±0.009 |

**Table 3.** Amplitude (mV) of ECG parameters in sport horses (N=30)

| Parameters | N1 (9) (Mean±SEM) | N2 (14) (Mean±SEM) | CD (7) (Mean±SEM) |
|---|---|---|---|
| P wave; Bifid | 0.20 | 0.40±0.07 | 0.40±0.03 |
| P wave; Biphasic | 0.48±0.06 | 0.40±0.05 | |
| Q Wave | 0.77±0.12 | 0.50±0.09 | 0.76±0.18 |
| R wave | 2.30±0.20 | 2.63±0.16 | 1.86±0.20 |
| S wave | 0.27±0.03 | 0.24±0.02 | 0.19±0.01 |
| QRS complex | 3.10±0.23 | 3.13±0.30 | 2.63±0.15 |
| Monophasic T wave (+) | 1.12±0.08 | 1.15±0.19 | 0.20±0.12 |
| Monophasic T wave (-) | 1.0 | 0 | 1.2 |
| Negative component of T | 0.47±0.18 | 0.71±0.10 | 0.73±0.18 |
| Positive component of T | 0.53±0.20 | 0.51±0.07 | 0.11±0.07 |

**Table 4.** The durations (seconds) in the ECG of the horses

| | ECG wave | | | | |
|---|---|---|---|---|---|
| Mean±SEM | P | QRS | T | PR | QT |
| N1 (n=9) | 0.14±0.006 | 0.12±0.002** | 0.12±0.02 | 0.35±0.01 | 0.50±0.01** |
| N2 (n=14) | 0.13±0.009 | 0.11±0.004 | 0.16±0.01 | 0.33±0.02 | 0.48±0.009 |
| CD (n=7) | 0.13±0.007 | 0.10±0.008** | 0.15±0.02 | 0.35±0.02 | 0.45±0.01** |

ECG parameters in the horses: N1 group (N1) age range between 3-5 years, N2 group (N2) age range between 6-17 years and CD group (CD) included 7 horses that the presence of any electrocardiogram changes (age range between 8-21 years). The difference of QRS wave and QT wave of the ECG between N1 group and CD group showed statistically significant,* represent $p \leq 0.01$.

## Discussion and Conclusion

As the cardiac output is the product of heart rate and stroke volume, reduction in heart rate reflects heart size and stroke volume [16]. In this study, the range of resting heart rate is approximately closed to that of the previous study conducted in Thoroughbred horses [17]. The

capability of Argentine Polo Pony horses and Thoroughbred horses in tolerating the intense exercises might be associated with their similar cardiovascular characteristic. QRS complex and QT interval of the electrocardiograms are other significant parameters in determination of the heart score and physiological fitness [16]. Our study showed statistically insignificant shorter QRS complex duration than those previously reported in other breeds such as Thoroughbred and Purebred Kurd horses [5,18]. Short QRS complex duration suggested that Argentine Polo Pony horses in our study groups had a smaller heart size than other sport horses. T wave is another parameter that can be used to determine the ventricular repolarization, inversion of the T wave was reported as a sign of myocardial ischemia and poor myocardium oxygenation [18]. Similarly, the current study in Kurd horses showed higher amplitude of negative component of T wave in CD group than in normal horses (N1 and N2 groups). This result suggested that CD group horses are more likely to have myocardial ischemia than in normal group of horses [5]. On the basis of this study, plasma cTnT assay could be a convenient tool for initial detection of myocardial dysfunction in horses. Based on our findings, it appears that measuring of the plasma cTnT is a good test, it would help to decide whether a horse with equivocal sign of exercise tolerance had or did not have myocardial disorder. The results of this study show that the commercial cTnT test kit is only accurate in identifying horses with high level of plasma cTnT (0.007). As illustrated in Fig. 2, the median plasma cTnT for CD group was 0.007 and the value was higher than the median concentration level in a group of normal horses. However, the stage of myocardial injury varies according to a high deviation in CD group. ECG abnormalities with plasma cTnT between 0.003-0.004 ng/ml might be grounds for suspecting minor myocardial injury but ascertaining this still required a further study. In conclusion, plasma cTnT levels correlate with the presence of abnormal ECG parameters in the sport horses, suggesting that it can be used a convenient assay to detect myocardial damage in the horses.

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