Manuscript Details

Manuscript number	JEVS_2019_177
Title	Comparison of two methods for 24-hour Holter monitoring in horses: evaluation of recording performance at rest and during exercise
Article type	Research paper

Abstract

The 24-hour Holter monitoring is the gold standard for diagnosing arrhythmias that occur intermittently or under exercise.The aim of this study was to compare two different methods for 24-hour Holter monitoring in horses, a 7-electrode system (7-ES) versus a 4-electrode system (4-ES), evaluating the recording performance at rest and during exercise. Six standardbred horses were included in the present prospective study. Two different methods for 24-hour Holter monitoring were used in each horse with a washout period of 1 week between each method of recording. In the first 15 minutes of the 24-hour Holter monitoring, a standard exercise test was performed. Holter recordings were analysed for: number of recorded hours; number of detached electrodes; total duration of artifacts over the15-minutes exercise. The number of recorded hours was significantly higher in the 7-ES (24 hours, range: 23-24 hours) in comparison to the 4-ES (6.5 hours, range: 1.2-20 hours; $P<0.05$). The number of detached electrodes was not significantly different among the two systems. The total duration of artifacts over the 15-minute exercise was significantly higher in the 7-ES (155 sec, range: 35-378 sec) in comparison to the 4-ES (25 sec, range: 10-32 sec; $P<0.05$). Our results showed a better recording performance during exercise using the 4-ES because of a lower number of artifacts. The 7-ES showed a better performance in terms of duration of the recording. In conclusion, we suggest using the 4-ES for exercise tests and the 7-ES if a longer ECG recording at rest is required.

Keywords	exercise, artifacts, electrocardiography, cardiology, equine.

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Pisa, 20th May 2019

To

Editor-in-Chief

Journal of Equine Veterinary Science

Dear Editor,

here is our paper titled “Comparison of two methods for 24-hour Holter monitoring in horses: evaluation of recording performance at rest and during exercise”, authored by Vezzosi et al. We would like to submit our paper for the publication on the Journal of Equine Veterinary Science as an Original Research Paper (Regular Paper).

This study was approved by the Ethical Committee (N. 45865/2016), University of Pisa and totally supported by funds from the University of Pisa. The manuscript has not been published or submitted for publication elsewhere. Authors’ contribution to the manuscript is equally distributed and no conflict of interest exists. All the authors have been approved the manuscript.

If further information is needed or you have any questions or requires, please do not hesitating to contact me.

Yours sincerely,

Dr. Rosalba Tognetti

HIGHLIGHTS

1) The aim was to compare two different methods for Holter monitoring in horses.
2) The 4-electrode system showed a better recording performance during exercise.
3) The 7-electrode system performs better when a long recording at rest is required.

Two methods for 24-hour Holter monitoring in horses: evaluation of recording performance at rest and during exercise

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Abstract

24-hour Holter monitoring is the gold standard for diagnosing arrhythmias that occur intermittently or under exercise, and it is a key component of equine cardiovascular examination. The aim of this study was to compare two different methods for 24-hour Holter monitoring in horses, a 7-electrode system (7-ES) versus a 4-electrode system (4-ES), assessing the recording performance at rest and during exercise.

Six standardbred horses were included in the present prospective study. Two different methods for 24-hour Holter monitoring were used in each horse with a washout period of one week between each recording method. In the first 15 minutes of the 24-hour Holter monitoring, a standard exercise test was performed. Holter recordings were analysed in terms of number of recorded hours; number of detached electrodes; total duration of artifacts over the 15-minute exercise.

The number of recorded hours was significantly higher in the 7-ES (24 hours, range: 23-24 hours) in comparison to the 4-ES (6.5 hours, range: 1.2-20 hours; $P<0.05$). The number of detached electrodes was not significantly different between the two systems. The total duration of artifacts over the 15-minute exercise was significantly higher in the 7-ES (155 sec, range: 35-378 sec) than in the 4-ES (25 sec, range: 10-32 sec; $P<0.05$).

Our results showed a better recording performance during exercise using the 4-ES due to the lower number of artifacts. The 7-ES showed a better performance in terms of recording duration. In conclusion, we suggest using the 4-ES for exercise tests, and the 7-ES when a longer ECG recording at rest is required.

Keywords: exercise, artifacts, electrocardiography, cardiology, equine.

1. Introduction

Electrocardiography (ECG) is the test of choice for the diagnosis of cardiac arrhythmias in horses, both at rest and during exercise [1]. A standard ECG is usually performed at rest using the base-apex lead placement [2]. A portable ECG unit is useful in the field for documenting arrhythmias. The reliability of a portable smartphone ECG device has been described in horses, cows and dogs [3-5] and "smart textile" electrodes have been tested to improve the quality of the signals recorded [6].

The limitations of a resting ECG arise largely because of the enormous cardiac reserve of the horse, which means that performance-limiting cardiac disease or abnormal rhythms during exercise rarely manifest themselves at rest [7]. Thus, ECG during exercise is an integral tool in the clinical evaluation of horses presented for episodes of exercise-associated collapse, decreased exercise tolerance, poor athletic performance, or cardiac murmurs [1,7-9].

Telemetry ECG recording and 24-hour Holter ECG monitoring are suitable for detecting heart rhythm disturbance at rest, as well as for assessing the presence of intermittent arrhythmias and for electrocardiographic evaluation during exercise [1,7,10-13]. An exercise ECG is often suitable to determine whether an arrhythmia has the potential to impair performance or could become a safety issue [1,14,15].

The critical points during 24-hour Holter ECG monitoring are the positioning of the electrodes, number of electrodes and leads used, and possible electrode detachment during exercise [16-18]. Several methods have been described for recording a 24-hour Holter monitoring in horses, using varying lead systems and a variable number of electrodes [7,10,19,20].

The aim of this study was to compare two different methods used in 24-hour Holter monitoring in horses, a 4-lead versus a 7-lead system, evaluating the recording performance both at rest and during exercise.

2. Materials and methods

The investigation was prospective and observational. A total of six healthy horses, owned by the Department of Veterinary Sciences of the University of Pisa, were enrolled in this study. The research protocol was approved by the Institutional Animal Care and Use Committee of the University of Pisa (permission number: 45865/2016). All the horses were considered healthy based on history, physical examination, electrocardiography, and echocardiography.

2.1. 24-hour Holter monitoring

Two different methods for 24-hour Holter monitoring were used in each horse with a washout period of one week between each recording method, for a total number of 12 recordings. In one method, the 24-hour Holter monitoring was performed using the 4-electrode system (4-ES) [7], in which the two positive electrodes were placed slightly caudal to the left cardiac apex, the negative electrode was placed over the left side of the thorax beside the withers, and the earth electrode was placed next to the negative electrode (**Fig. 1**).

In the other method, a 7-electrode system (7-ES) was used [19], in which three positive electrodes and the earth electrode were placed over the left cardiac apex behind the olecranon, and three negative electrodes were placed on the right precordial area behind the olecranon, symmetrically to the left side but with one electrode less (**Fig. 2**). On the left side, the electrodes were placed in vertical order,

from ventral to dorsal, as follows: channel 3+, channel 1+, earth, channel 2+. On the right side, the electrodes were placed in vertical order, from ventral to dorsal, as follows: channel 3-, channel 2-, channel 1-.

With both methods, disposable contact electrodes were used (F 50 SG, EF medica Srl, Italy). The electrodes were placed after shaving the positioning area, applying a drop of ECG gel in the center of the electrode and using a small amount of glue to fix the electrode on the skin.

The digital Holter recorder (ClickHolter, Cardioline, Italy) was connected to the electrodes and a short ECG was visualized on a PC monitor to check its quality, then the recording was started. The Holter device (recorder and cables) was fixed to the animal using an elastic band (Vetrap, 3M Italia Srl, Italy), over which an adhesive band (Tensoplast, Sixtus, Italy) was placed. The whole device was protected by a saddle pad.

2.2. Standardized exercise tests

In the first 15 minutes of the 24-hour Holter monitoring, a standard exercise test was performed. The horses were lunged for 7.5 minutes in a clockwise circle and then for 7.5 minutes in a counter-clockwise circle. Regarding exercise intensity, all horses were moved at a trot velocity. After the 15-minute exercise test, horses were housed in single 4x4 meters boxes for the remaining hours of the Holter monitoring.

2.3. Holter monitoring analysis

At 24 hours the Holter recorder was removed and the recorded data were downloaded onto a PC and processed with a specific software program (CubeHolter, Cardioline, Italy). In a blinded fashion, the Holter recordings were reviewed by one

expert operator (T.V.), who subjectively assessed the ECG tracing quality. Holter recording were analysed for: 1) number of recorded hours over the 24-hour recording (H); 2) number of detached electrodes (DE) at the end of the 24-hour period; 3) percentage of detached electrodes in relation to the number of electrodes applied (detached electrodes divided by the number of applied electrodes and multiplied by 100) (%DE); 4) number of total artifacts (TA) during the 15-minute exercise; 5) percentage TA during the 15-minute exercise (duration of TA expressed in seconds, divided by 900 seconds and multiplied by 100 (%TA). Artifacts were defined as recording segments in which P waves and/or QRS complexes were not identified [4,21].

2.4. Statistical analysis

Descriptive statistics were generated. The normality of data distribution was tested by the Kolmogorov-Smirnov test, and parametric or nonparametric tests were used according to the Gaussian distribution. According to data distribution, a Mann-Whitney test was used to evaluate the differences in the variables analysed (DE, H, TA, %TA and %DE) between the two groups (7-ES versus 4-ES). Data are reported as median and range (minimum–maximum), unless otherwise stated.
Statistical analyses were performed with GraphPad Prism 6 (USA). A *P* value of <0.05 was considered significant.

3. Results

3.1. Animals

A total of six standard-bred female horses were included in the study. The median age was 9.5 years, with a range between 8 and 13 years. The median body weight was 461 kg (range: 353-656 Kg) and the median BCS was 3/5 (range: 3-4/5) [22]. The results of the 24-hour Holter analysis are reported in Table 1. The number of recorded hours was significantly higher in the 7-ES than in the 4-ES. The total duration of artifacts and the percentage of artifacts over the 15- minute exercise was significantly higher in the 7-ES in comparison to the 4-ES. No electrodes had become detached at the end of the exercise test in any horse.

4. Discussion

Long-term ECG recording is the gold standard for diagnosing arrhythmias that occur intermittently and/or under exercise, and is a key component of equine cardiovascular examination [1]. Several methods of 24-hour Holter monitoring have been proposed in the horse, using from 4 to 7 electrodes, placed only on the left side of the thorax or on both sides [7,10,19,20]. The critical points during 24-hour Holter monitoring are motion artifacts and possible electrode detachment during exercise, which prevent a reliable interpretation of the ECG trace [16,17].

Our results showed a better performance of the 4-ES during exercise because of the lower number of artifacts in the recording. On the other hand, the 7-ES showed a better performance during long-term recording at rest.

The number of recorded hours was significantly higher in the 7-ES than in the 4-ES. In fact, a median of 24 hours was recorded using the 7-ES. On the other hand, the median duration of the recording was 6.5 hours using the 4-ES, with a 20-hour duration of the recording obtained only in one horse. This performance difference stems from the problem of electrode detachment.

In our study, the maximum number of detached electrodes was two for both methods. In the 7-ES, 3 electrocardiographic channels are simultaneously recorded from three independent bipolar leads. Thus, even if one or two electrodes become detached, at least one channel continues to record. In the 4-ES, 2 electrocardiographic channels are available for interpretation. However, if the dorsal negative electrode becomes detached, there are no remaining channels, and in our experience the dorsal electrodes become more frequently detached with the 4-ES. This is the reason for the significant lower number of recorded hours with this system. In the 4-ES, it is difficult to apply a protective bandage to the dorsal electrodes because of the positioning that is very close to the withers, which is an area highly susceptible to neck movements.

Another possible advantage of using a 7-ES with three independent bipolar leads placed on both sides of the chest is that each lead detects the potential difference between its two electrodes from just one angle, which might help to differentiate between a normal or abnormal complex [20,23]. This is more difficult with the 4-ES in which only a base-apex angle can be studied.

Although the 7-ES showed a good performance in long-term recording at rest, the total artifacts and the percentage of artifacts over the 15-minute exercise were significantly higher in the 7-ES than in the 4-ES. A possible explanation is that in the 7-ES, the electrodes are placed very close to the thoracic limbs, which probably

influenced the quality of the recording when the horse is exercising. In the 4-ES, the two positive electrodes are placed farther away from the thoracic limb in comparison to the 7-ES, which probably reduces the movement artifacts during exercise. It is known that the problems related to motion artifacts in the equine ECG become more significant during exercise [24,25]. This is why the 4-ES has been proposed as a modification of the base-apex lead used for standard ECG at rest, and adapted for the electrocardiographic recording during exercise by minimizing the number of motion artifacts [7].

5. Conclusions

Based on these results we suggest using the 4-ES for ECG recording during exercise and the 7-ES when a longer ECG recording at rest is required. Further studies are needed to evaluate the diagnostic performance of different lead systems for Holter monitoring in horses with arrhythmias.

Funding

This study was supported by funds from the University of Pisa.

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Tables

Table 1. Results of the 24-Holter monitoring with the two lead systems.

H (hours)		DE (n°)		DE (%)		TA (sec)		TA (%)	
7-ES	4-ES	7-ES	4-ES	7-ES	4-ES	7-ES	4-ES	7-ES	4-ES
24 (23-24)	6.5 (1.2-20)*	0 (0-2)	2 (0-2)	0 (0-29)	50 (0-50)	155 (35-378)	25 (10-32)*	14 (4-42)	3 (1-6)*

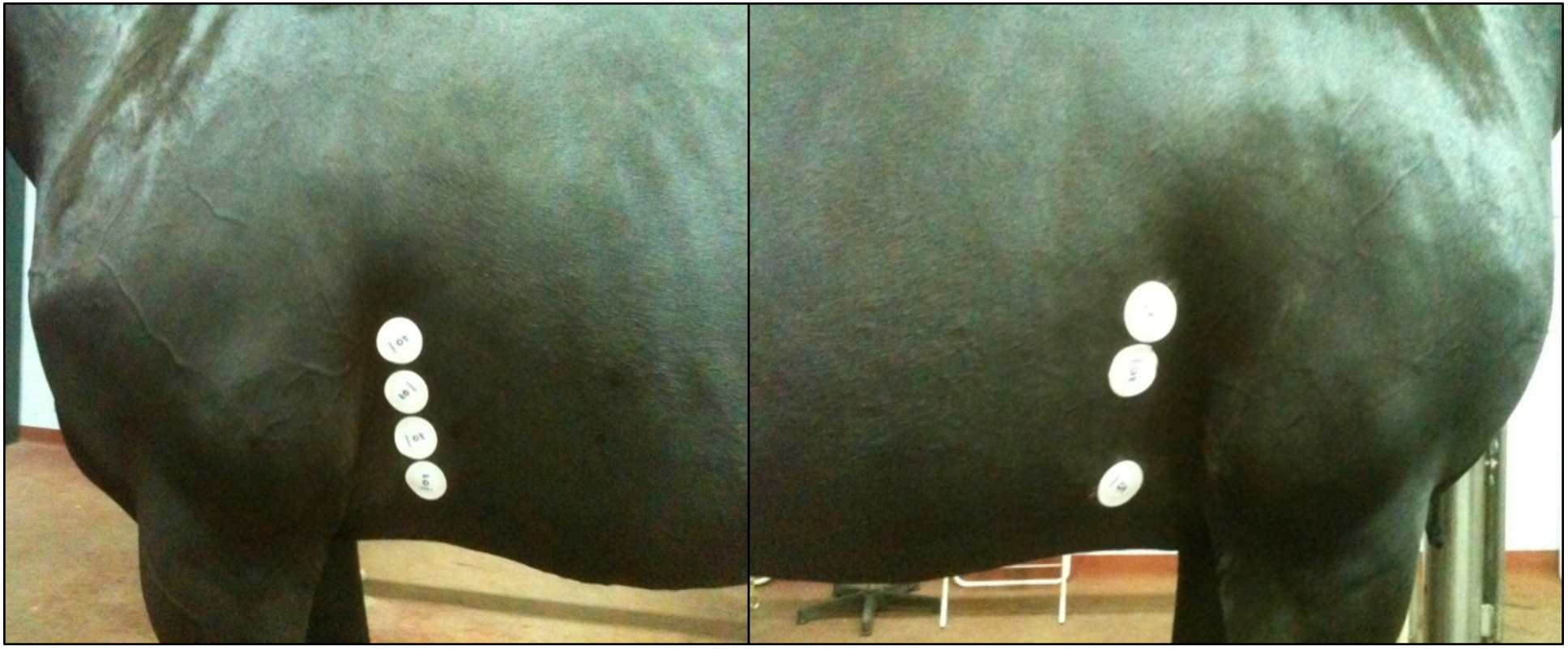
H, number of recorded hours over the 24-hour recording; DE (n°), number of detached electrodes at the end of the 24-hour period; DE (%), percentage of detached electrodes in relation to the number of electrodes applied; TA (sec), number of total artifacts during the 15-minute exercise; TA (%) percentage of total artifacts during the 15-minute exercise; 7-ES, 7-electrode system; 4-electrode system.

*P<0.05 in comparison with the 7-ES.

Figure legends

Fig. 1. Positioning of the electrodes in the 4-electrode system for 24-Holter monitoring.

Fig.2. Positioning of the electrodes in the 7-electrode system for 24-Holter monitoring.



CONFLICT OF INTEREST

Dear Editor,

The Authors' contribution to the manuscript is equally distributed and no conflict of interest exists.

Yours sincerely,

Dr. Rosalba Tognetti

ETHICAL STATEMENT

Dear Editor,

this *in vivo* study was approved by the Institutional Animal Care and Use Committee of the University of Pisa (D.R. prot. N. 45865/2016). The University of Pisa owned the horses included in the study.

Yours sincerely,

Dr. Rosalba Tognetti