

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 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 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

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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.

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3 **1 Two methods for 24-hour Holter monitoring in horses: evaluation of recording**
4 **2 performance at rest and during exercise**
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63 **Abstract**
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65 24-hour Holter monitoring is the gold standard for diagnosing arrhythmias that occur
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67 intermittently or under exercise, and it is a key component of equine cardiovascular
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69 examination. The aim of this study was to compare two different methods for 24-hour
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71 Holter monitoring in horses, a 7-electrode system (7-ES) versus a 4-electrode system
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73 (4-ES), assessing the recording performance at rest and during exercise.
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76 Six standardbred horses were included in the present prospective study. Two
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78 different methods for 24-hour Holter monitoring were used in each horse with a
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80 washout period of one week between each recording method. In the first 15 minutes
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82 of the 24-hour Holter monitoring, a standard exercise test was performed. Holter
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84 recordings were analysed in terms of number of recorded hours; number of detached
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86 electrodes; total duration of artifacts over the 15-minute exercise.
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89 The number of recorded hours was significantly higher in the 7-ES (24 hours, range:
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91 23-24 hours) in comparison to the 4-ES (6.5 hours, range: 1.2-20 hours; $P < 0.05$).
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94 The number of detached electrodes was not significantly different between the two
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96 systems. The total duration of artifacts over the 15-minute exercise was significantly
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98 higher in the 7-ES (155 sec, range: 35-378 sec) than in the 4-ES (25 sec, range: 10-
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100 32 sec; $P < 0.05$).

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102 Our results showed a better recording performance during exercise using the 4-ES
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104 due to the lower number of artifacts. The 7-ES showed a better performance in terms
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106 of recording duration. In conclusion, we suggest using the 4-ES for exercise tests,
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108 and the 7-ES when a longer ECG recording at rest is required.
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112 **Keywords:** exercise, artifacts, electrocardiography, cardiology, equine.
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123 **45 1. Introduction**
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125 46 Electrocardiography (ECG) is the test of choice for the diagnosis of cardiac
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127 47 arrhythmias in horses, both at rest and during exercise [1]. A standard ECG is usually
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129 48 performed at rest using the base-apex lead placement [2]. A portable ECG unit is
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131 49 useful in the field for documenting arrhythmias. The reliability of a portable
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133 50 smartphone ECG device has been described in horses, cows and dogs [3-5] and
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135 51 “smart textile” electrodes have been tested to improve the quality of the signals
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137 52 recorded [6].
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140 53 The limitations of a resting ECG arise largely because of the enormous cardiac
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142 54 reserve of the horse, which means that performance-limiting cardiac disease or
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144 55 abnormal rhythms during exercise rarely manifest themselves at rest [7]. Thus, ECG
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146 56 during exercise is an integral tool in the clinical evaluation of horses presented for
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148 57 episodes of exercise-associated collapse, decreased exercise tolerance, poor
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150 58 athletic performance, or cardiac murmurs [1,7-9].
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153 59 Telemetry ECG recording and 24-hour Holter ECG monitoring are suitable for
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155 60 detecting heart rhythm disturbance at rest, as well as for assessing the presence of
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157 61 intermittent arrhythmias and for electrocardiographic evaluation during exercise
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159 62 [1,7,10-13]. An exercise ECG is often suitable to determine whether an arrhythmia
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161 63 has the potential to impair performance or could become a safety issue [1,14,15].
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164 64 The critical points during 24-hour Holter ECG monitoring are the positioning of the
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166 65 electrodes, number of electrodes and leads used, and possible electrode detachment
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168 66 during exercise [16-18]. Several methods have been described for recording a 24-
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170 67 hour Holter monitoring in horses, using varying lead systems and a variable number
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172 68 of electrodes [7,10,19,20].
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183 69 The aim of this study was to compare two different methods used in 24-hour Holter
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185 70 monitoring in horses, a 4-lead versus a 7-lead system, evaluating the recording
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187 71 performance both at rest and during exercise.
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191 192 73 **2. Materials and methods**

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194 74 The investigation was prospective and observational. A total of six healthy horses,
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196 75 owned by the Department of Veterinary Sciences of the University of Pisa, were
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198 76 enrolled in this study. The research protocol was approved by the Institutional Animal
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200 77 Care and Use Committee of the University of Pisa (permission number: 45865/2016).
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202 78 All the horses were considered healthy based on history, physical examination,
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204 79 electrocardiography, and echocardiography.
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208 209 81 *2.1. 24-hour Holter monitoring*

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211 82 Two different methods for 24-hour Holter monitoring were used in each horse with a
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213 83 washout period of one week between each recording method, for a total number of
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215 84 12 recordings. In one method, the 24-hour Holter monitoring was performed using
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217 85 the 4-electrode system (4-ES) [7], in which the two positive electrodes were placed
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219 86 slightly caudal to the left cardiac apex, the negative electrode was placed over the
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221 87 left side of the thorax beside the withers, and the earth electrode was placed next to
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223 88 the negative electrode (**Fig. 1**).

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226 89 In the other method, a 7-electrode system (7-ES) was used [19], in which three
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228 90 positive electrodes and the earth electrode were placed over the left cardiac apex
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230 91 behind the olecranon, and three negative electrodes were placed on the right
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232 92 precordial area behind the olecranon, symmetrically to the left side but with one
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234 93 electrode less (**Fig. 2**). On the left side, the electrodes were placed in vertical order,
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243 94 from ventral to dorsal, as follows: channel 3+, channel 1+, earth, channel 2+. On the
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245 95 right side, the electrodes were placed in vertical order, from ventral to dorsal, as
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247 96 follows: channel 3-, channel 2-, channel 1-.

249 97 With both methods, disposable contact electrodes were used (F 50 SG, EF medica
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252 98 Srl, Italy). The electrodes were placed after shaving the positioning area, applying a
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254 99 drop of ECG gel in the center of the electrode and using a small amount of glue to fix
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256 100 the electrode on the skin.

258 101 The digital Holter recorder (ClickHolter, Cardioline, Italy) was connected to the
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260 102 electrodes and a short ECG was visualized on a PC monitor to check its quality, then
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262 103 the recording was started. The Holter device (recorder and cables) was fixed to the
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264 104 animal using an elastic band (Vetrap, 3M Italia Srl, Italy), over which an adhesive
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266 105 band (Tensoplast, Sixtus, Italy) was placed. The whole device was protected by a
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268 106 saddle pad.

273 108 *2.2. Standardized exercise tests*

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275 109 In the first 15 minutes of the 24-hour Holter monitoring, a standard exercise test was
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277 110 performed. The horses were lunged for 7.5 minutes in a clockwise circle and then for
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279 111 7.5 minutes in a counter-clockwise circle. Regarding exercise intensity, all horses
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281 112 were moved at a trot velocity. After the 15-minute exercise test, horses were housed
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283 113 in single 4x4 meters boxes for the remaining hours of the Holter monitoring.

288 115 *2.3. Holter monitoring analysis*

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290 116 At 24 hours the Holter recorder was removed and the recorded data were
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292 117 downloaded onto a PC and processed with a specific software program (CubeHolter,
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294 118 Cardioline, Italy). In a blinded fashion, the Holter recordings were reviewed by one

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303 119 expert operator (T.V.), who subjectively assessed the ECG tracing quality. Holter
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305 120 recording were analysed for: 1) number of recorded hours over the 24-hour recording
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307 121 (H); 2) number of detached electrodes (DE) at the end of the 24-hour period; 3)
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309 122 percentage of detached electrodes in relation to the number of electrodes applied
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311 123 (detached electrodes divided by the number of applied electrodes and multiplied by
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313 124 100) (%DE); 4) number of total artifacts (TA) during the 15-minute exercise; 5)
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315 125 percentage TA during the 15-minute exercise (duration of TA expressed in seconds,
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317 126 divided by 900 seconds and multiplied by 100 (%TA). Artifacts were defined as
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319 127 recording segments in which P waves and/or QRS complexes were not identified
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321 128 [4,21].
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327 130 *2.4. Statistical analysis*

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329 131 Descriptive statistics were generated. The normality of data distribution was tested by
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331 132 the Kolmogorov-Smirnov test, and parametric or nonparametric tests were used
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333 133 according to the Gaussian distribution. According to data distribution, a Mann-
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335 134 Whitney test was used to evaluate the differences in the variables analysed (DE, H,
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337 135 TA, %TA and %DE) between the two groups (7-ES versus 4-ES). Data are reported
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339 136 as median and range (minimum–maximum), unless otherwise stated.

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341 137 Statistical analyses were performed with GraphPad Prism 6 (USA). A *P* value of
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343 138 <0.05 was considered significant.
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348 140 **3. Results**

349 350 141 *3.1. Animals*

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

150

151 **4. Discussion**

152 Long-term ECG recording is the gold standard for diagnosing arrhythmias that occur
153 intermittently and/or under exercise, and is a key component of equine
154 cardiovascular examination [1]. Several methods of 24-hour Holter monitoring have
155 been proposed in the horse, using from 4 to 7 electrodes, placed only on the left side
156 of the thorax or on both sides [7,10,19,20]. The critical points during 24-hour Holter
157 monitoring are motion artifacts and possible electrode detachment during exercise,
158 which prevent a reliable interpretation of the ECG trace [16,17].
159 Our results showed a better performance of the 4-ES during exercise because of the
160 lower number of artifacts in the recording. On the other hand, the 7-ES showed a
161 better performance during long-term recording at rest.

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162 The number of recorded hours was significantly higher in the 7-ES than in the 4-ES.
163 In fact, a median of 24 hours was recorded using the 7-ES. On the other hand, the
164 median duration of the recording was 6.5 hours using the 4-ES, with a 20-hour
165 duration of the recording obtained only in one horse. This performance difference
166 stems from the problem of electrode detachment.
167 In our study, the maximum number of detached electrodes was two for both
168 methods. In the 7-ES, 3 electrocardiographic channels are simultaneously recorded
169 from three independent bipolar leads. Thus, even if one or two electrodes become
170 detached, at least one channel continues to record. In the 4-ES, 2
171 electrocardiographic channels are available for interpretation. However, if the dorsal
172 negative electrode becomes detached, there are no remaining channels, and in our
173 experience the dorsal electrodes become more frequently detached with the 4-ES.
174 This is the reason for the significant lower number of recorded hours with this system.
175 In the 4-ES, it is difficult to apply a protective bandage to the dorsal electrodes
176 because of the positioning that is very close to the withers, which is an area highly
177 susceptible to neck movements.
178 Another possible advantage of using a 7-ES with three independent bipolar leads
179 placed on both sides of the chest is that each lead detects the potential difference
180 between its two electrodes from just one angle, which might help to differentiate
181 between a normal or abnormal complex [20,23]. This is more difficult with the 4-ES in
182 which only a base-apex angle can be studied.
183 Although the 7-ES showed a good performance in long-term recording at rest, the
184 total artifacts and the percentage of artifacts over the 15-minute exercise were
185 significantly higher in the 7-ES than in the 4-ES. A possible explanation is that in the
186 7-ES, the electrodes are placed very close to the thoracic limbs, which probably

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

195

196 **5. Conclusions**

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

201

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204

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278 **Tables**

279 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)*

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281 H, number of recorded hours over the 24-hour recording; DE (n°), number of detached electrodes at the end of
282 the 24-hour period; DE (%), percentage of detached electrodes in relation to the number of electrodes applied;
283 TA (sec), number of total artifacts during the 15-minute exercise; TA (%) percentage of total artifacts during the
284 15-minute exercise; 7-ES, 7-electrode system; 4-electrode system.

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

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287 **Figure legends**

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

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





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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

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3 **ETHICAL STATEMENT**
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7 Dear Editor,
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11 this *in vivo* study was approved by the Institutional Animal Care and Use Committee of the
12 University of Pisa (D.R. prot. N. 45865/2016). The University of Pisa owned the horses
13 included in the study.
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20 Yours sincerely,
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22 Dr. Rosalba Tognetti
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