

Manuscript Details

Manuscript number	JEVS_2018_29_R1
Title	Evaluation of a smartphone electrocardiograph in healthy horses: comparison with standard base-apex electrocardiography
Article type	Research paper

Abstract

A good diagnostic accuracy of smartphone-based electrocardiography in the evaluation of heart rate, heart rhythm and ECG measurements has been reported in humans, dogs and cats. The aim of this study was to assess the feasibility of smartphone-based electrocardiography in horses. Fifty healthy adult horses were enrolled. Standard base-apex ECG and smartphone ECG were simultaneously recorded in each horse. All ECGs were reviewed by one blinded operator, who judged whether tracings were acceptable for interpretation and performed electrocardiographic measurements. Agreement between smartphone and standard base-apex ECG in the analysis of tracings was evaluated. Smartphone ECG tracings were interpretable in 48/50 (96%) cases. A perfect agreement between smartphone and standard ECG tracings was found in the assessment of heart rate. Heart rate automatically measured by the smartphone application was not reliable. In terms of electrocardiographic waves and interval duration, minimal differences of no clinical value were found between smartphone and standard ECG. Agreement was found for QRS complex polarity evaluation, but not for P wave polarity. Baseline artefacts were rare but significantly higher in the smartphone ECG tracings compared to standard ECG. The smartphone ECG can record single-lead ECG tracings of an adequate quality for interpretation in horses. The smartphone ECG could represent an additional tool for the electrocardiographic evaluation of horses, but is not a substitute for the standard base-apex ECG.

Keywords	Equine; Cardiology; Electrocardiography; Smartphone; Validation
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Suggested reviewers	Josefa Fernandez-del Palacio, Oriol Domenech, ALBERTO TARDUCCI

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Pisa, 4th March 2018

To

Editor-in-Chief

Journal of Equine Veterinary Science

Dear Editor,

here is our paper titled “**Evaluation of a smartphone electrocardiograph in horses: comparison with standard base-apex electrocardiography**”, authored by Vezzosi et al. and revised according to the reviewers’ comments and suggestions (JEVS_2018_29).

This study was approved by the Ethical Committee (N. 45965/2016), 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. Tommaso Vezzosi

REPLIES TO REVIEWERS

The authors (AU) would like to thank the reviewers (RW) for taking the time to review this manuscript. We found the comments appropriate and very useful. All the points have been addressed and corrections have been made to the manuscript accordingly. The changes to the manuscript are highlighted in yellow.

The replies to the reviewers' comments are reported below.

Reviewer 1 (yellow)

RW: Change the title adding “healthy”

AU: Done (see line 1).

RW: “Specify in which period of the year the ECG recording was performed...”

AU: Done (see lines 72-73).

RW: “Did the authors use ECG filters?”

AU: Done (see lines 90-91).

RW: “...How did you choose the position? Do you always used the same intercostal space?”

AU: Done (see lines 96-97).

RW: Line 146. Remove references from the results.

AU: Removed.

RW: Line 182. Modify in “QRS complex duration measured on standard and smartphone ECG tracings showed a bias of...”.

AU: Done (see lines 189-190).

RW: Line 162. Would you consider if these results would have been different in case the amplitude used in the study would have been 10 mm=1 mV rather than 20mm=1mV for the Smartphone ECG?

AU: Thank you very much for your interesting comment. During the study design we decide to use the 20mm/mV setting because using 10mm/mV the amplitude of the QRS complexes was very low on the smartphone screen in most horses and the application did not display the heart rate in these cases. If the application does not detect the heart rate, it does not record the ECG. Thus, we decided to record all ECG at 20 mm/mV to standardize the recording in the study and to obtain the recording in most horses.

RW: Line 236: please modify “The smartphone ECG can record single-lead ECG tracings of a good quality in healthy horses”.

AU: Done (see line 244).

RW: Fig. 1: The P wave polarity in this image seems to be negative...”.

AU: In our opinion, the P wave in the figure 1 shows both a negative (beginning) and a positive (ending) component. So, it can be defined as “biphasic”. It is not completely “negative”. The P wave had this biphasic morphology in most smartphone ECG tracings (75%). Thus, we choose to show this figure in the manuscript.

RW: Fig. 2: Please provide the ECG tracing with a paper speed = 25mm/sec as it has been described in Materials and Methods.

AU: Thank you very much for your comment. The paper speed of figure 2 is at 25 mm/sec as reported in the M&M. There was a mistake in the figure caption. We modified accordingly (see line 328).

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62
63 **Reviewer 2 (green)**

64 RW: Introduction. Line 67: ... and ECG measurements compared with conventional ECG.

65 **AU: The sentence has been added to the main text (see line 67).**

66
67
68 RW: Material and methods. Line 70: Were all the horses owned by the university or the
69 owners of the department?

70 **AU: Yes, all the horses were property of the Department of Veterinary Sciences,**
71 **University of Pisa.**

72
73 RW: Line 80-81: How were the electrodes of conventional ECG placed on the skin? Please,
74 indicate whether they were alligator clips, discs....

75 **AU: The placement of the electrodes has been better clarified in the text (see line 82).**

76
77 RW: Line 98-99: I suggest to move this sentence after line 83.

78 **AU: The sentences have been reorganized (see lines 87-89 and 103-104).**

79
80
81 RW: How were the ECG recordings synchronized with both methods? And, ECG
82 measurements? How did you select the same cardiac cycles for measurements? Please
83 indicate. It is not clear.

84 **AU: As indicated at lines 92-93, the smartphone ECG tracing was simultaneously**
85 **recorded, starting and ending at the same time as the standard ECG. This was possible**
86 **because the smartphone ECG started storing the tracing when the applications began to**
87 **show the HR on the screen, thus at that time a second operator began to store the**
88 **tracings on the standard ECG machine. Using this technique, we were sure that both**
89 **recordings were stored simultaneously.**

90
91 RW: Line 117: normal range is most appropriate for HR than “normal”.

92 **AU: Modified accordingly (see lines 124-126).**

93
94
95 RW: Results. Line 186. It would be interesting for readers to include a figure of the most
96 common artifacts recorded by smartphone. Also, a figure of two tracings obtained by
97 conventional ECG and smartphone showing different polarity of P waves.

98 **AU: Thanks for the suggestion. We added both the images requested (see figures 3 and**
99 **4).**

100
101 RW: Line 187. A question: You have attempted to improve the contact of the electrodes of
102 the smartphone in horses in which they have obtained artefacts? Please clarify.

103 **AU: Yes. In cases with many artefacts we tried to improve the contact of the electrodes**
104 **and the quality of ECG signal on the smartphone using more alcohol or slight modifying**
105 **the orientation of the smartphone. However, these attempts were not useful in most**
106 **cases since the artefacts were due to skin or limb movements.**

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HIGHLIGHTS

- 1) The aim was to assess the feasibility of smartphone electrocardiography in horses.
- 2) The smartphone device could record good quality ECG tracings in 96% of horses.
- 3) Reliable heart rate was obtained when manually measured on digitized tracings.
- 4) The smartphone device was reliable for measurement of ECG wave and intervals.
- 5) The smartphone ECG can be useful for electrocardiographic screening in horses.

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4 1 **Evaluation of a smartphone electrocardiograph in **healthy** horses: comparison**
5
6 2 **with standard base-apex electrocardiography**
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63 **21 Abstract**
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66 22 A good diagnostic accuracy of smartphone-based electrocardiography in the
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68 23 evaluation of heart rate, heart rhythm and ECG measurements has been reported in
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70 24 humans, dogs and cats. The aim of this study was to assess the feasibility of
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72 25 smartphone-based electrocardiography in horses.

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74 26 Fifty healthy adult horses were enrolled. Standard base-apex ECG and smartphone
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76 27 ECG were simultaneously recorded in each horse. All ECGs were reviewed by one
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78 28 blinded operator, who judged whether tracings were acceptable for interpretation and
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80 29 performed electrocardiographic measurements. Agreement between smartphone and
81
82 30 standard base-apex ECG in the analysis of tracings was evaluated.

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84 31 Smartphone ECG tracings were interpretable in 48/50 (96%) cases. A perfect
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86 32 agreement between smartphone and standard ECG tracings was found in the
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88 33 assessment of heart rate. Heart rate automatically measured by the smartphone
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90 34 application was not reliable. In terms of electrocardiographic waves and interval
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92 35 duration, minimal differences of no clinical value were found between smartphone
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94 36 and standard ECG. Agreement was found for QRS complex polarity evaluation, but
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96 37 not for P wave polarity. Baseline artefacts were rare but significantly higher in the
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98 38 smartphone ECG tracings compared to standard ECG.

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100 39 The smartphone ECG can record single-lead ECG tracings of an adequate quality for
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102 40 interpretation in horses. The smartphone ECG could represent an additional tool for
103
104 41 the electrocardiographic evaluation of horses, but is not a substitute for the standard
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106 42 base-apex ECG.
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111 43
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113 44 **Keywords:** Equine; Cardiology; Electrocardiography; Smartphone; Validation.
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123 **45 Introduction**
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125 46 In equine species, surface electrocardiography (ECG) is the gold standard for the
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127 47 diagnosis of arrhythmias, both at rest and during exercise [1]. In horses, a standard
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129 48 ECG is usually performed at rest using the base-apex lead placement [2]. The
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131 49 procedure requires an electrocardiographic machine, cables and electrodes.
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133 50 Therefore, a standard ECG is not always practical in emergency and sports
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135 51 medicine. Telemetric systems and Holter monitoring are used in horses for ECG
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137 52 recordings during and post exercise [3]. However, this technology can be expensive
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139 53 and requires the use of cables and electrodes, and a recorder is usually fixed on the
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141 54 horse with bandages or dedicated vests. A wireless, smartphone-based technology
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143 55 for ECG recording could be an attractive complementary tool for electrocardiographic
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145 56 screening in horses.

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148 57 In humans, smartphone devices recording a one-lead ECG tracing have been
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150 58 developed using specific adaptors and software [4-10]. Several studies have
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152 59 highlighted the accuracy of smartphone ECG tracings in measuring heart rate (HR),
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154 60 evaluating heart rhythm [11-18] and detecting ECG changes associated with
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156 61 myocardial ischemia [19,20].

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158 62 Similarly, smartphone ECG technology can provide adequate tracings, with an
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160 63 accurate assessment of HR and heart rhythm in dogs and cats [21,22].

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162 64 To the best of the authors' knowledge, no studies on the use of smartphone ECG in
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164 65 the horse have been performed. The present study thus assessed the feasibility of
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166 66 smartphone-based electrocardiography in horses, and its accuracy to evaluate HR
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168 67 and ECG measurements **compared with conventional ECG**.
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174 **69 Materials and methods**
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183 70 *Animals*
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185 71 A total of 50 healthy horses owned by the Department of Veterinary Sciences of the
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187 72 University of Pisa, were enrolled in this study. The study period was from March 2016
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189 73 to November 2017. The research protocol was approved by the Institutional Animal
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192 74 Care and Use Committee of the University of Pisa (45965/2016). All the horses were
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194 75 considered healthy based on history, physical examination, electrocardiography and
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196 76 echocardiography.
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200 78 *ECG acquisition and analysis*
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202 79 After the horses had been familiarized with the examination room, a standard base-
203
204 80 apex ECG (MAC 1600 ECG system, GE Healthcare, USA) was acquired for 30
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206 81 seconds in conscious, non-sedated horses in standing position. Surface electrodes
207
208 82 were attached to the skin by alligator clips. The left arm electrode (positive) was
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210 83 positioned at the cardiac apex, the right arm electrode (negative) was placed two
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212 84 thirds of the way down the right jugular groove, and the third electrode was placed at
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214 85 any site remote from the heart. Lead I was selected to record the ECG [23].
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217 86 No clipping was performed, and only alcohol was rubbed on the skin to maintain
218
219 87 electrical contact. The same operator (C.B.) always recorded the standard ECG. For
220
221 88 each horse, the ECG tracings obtained with the standard base-apex method were
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223 89 printed at a paper speed of 25 mm/s with a gain of 5 mm/mV. A sampling frequency
224
225 90 of 1000 Hz for standard ECG acquisition was used, with a 100 Hz low-pass filter and
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227 91 a 0.3–0.5 Hz high-pass filter.
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230 92 A smartphone ECG tracing was simultaneously recorded, starting and ending at the
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232 93 same time as the standard ECG, using a single-lead bipolar ECG (AliveCor
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234 94 Veterinary Heart Monitor, AliveCor, USA) and its software interface (AliveECG Vet,
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243 95 AliveCor, USA), as previously described [22]. The smartphone ECG tracings were
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245 96 recorded with an iPhone 5S (Apple, USA). The smartphone device was placed on the
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247 97 left chest wall at the level of the olecranon (precordial area). A dorso-ventral
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249 98 orientation with a 30° cranial inclination of the smartphone case was used in each
250
251 99 horse, with the camera side of the smartphone located ventrally (Fig. 1). A small
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253 100 amount of alcohol was rubbed on the left precordial area to obtain a good quality
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255 101 smartphone ECG signal. Smartphone ECG recordings were automatically digitized
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257 102 by the device, sent via email and stored as a PDF (Fig. 2). The same operator (M.P.)
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259 103 recorded all the smartphone ECGs. The same operator (M.P.) recorded all the
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261 104 smartphone ECGs.

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264 105 For each horse, the ECG tracings obtained with the standard base-apex method
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266 106 were printed at a paper speed of 25 mm/s with a gain of 5 mm/mV, while the ECG
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268 107 tracings obtained with the smartphone ECG, were printed at a paper speed of 25
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270 108 mm/s with a gain of 20 mm/mV.

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273 109 In a blinded fashion, all smartphone ECG tracings were reviewed by one expert
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275 110 operator (T.V.) to judge whether the tracings were acceptable for interpretation.
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277 111 Tracings were considered acceptable for interpretation if baseline artifacts were
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279 112 absent for at least 80% of each tracing. Baseline artifacts were defined as ECG
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281 113 segments in which P waves and/or QRS complexes could not be identified.

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283 114 The same operator (T.V.) performed ECG measurements on all tracings, using lead I
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285 115 of the standard ECG and the only available lead of the smartphone ECG.

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288 116 The following variables were measured from both ECG tracings for each horse: HR
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290 117 (bpm), duration of P wave (ms), PR interval (ms), QRS complex (ms), and QT
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292 118 interval (ms). Each wave or interval duration was calculated using three randomly
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294 119 selected heartbeats and the mean of the three measurements was used for statistical

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302
303 120 analysis. The mean HR was calculated as the mean value of three independent HR
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305 121 calculations from three different areas on the ECG tracings. The number of QRS
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307 122 complexes was counted over six seconds and multiplied by ten to calculate the HR
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309 123 per minute (bpm). The mean HR calculated automatically by the smartphone
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311 124 application (App HR) was also recorded. The normal range for HR was defined as
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313 125 between 28 and 44 bpm [2]. Bradycardia was defined when the HR was below 28
314
315 126 bpm, and tachycardia when the HR was greater than 44 bpm [2]. P wave and QRS
316
317 127 complex polarity were evaluated. Presence (yes, no) and duration (ms) of baseline
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319 128 artifacts were assessed.
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324 130 *Statistical analysis*

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326 131 The analysis was performed only with paired ECG tracings that were acceptable for
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328 132 interpretation, and the standard ECG was set as the reference method. Cohen's κ
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330 133 test was used to calculate the agreement between the smartphone ECG and
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332 134 standard ECG for HR classification (normal, bradycardia, tachycardia), P wave
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334 135 (positive, negative, biphasic) and QRS polarity (positive, negative). The κ coefficient
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336 136 was interpreted as follows: values ≤ 0.20 as no agreement, 0.21–0.40 as fair, 0.41–
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338 137 0.60 as moderate, 0.61–0.80 as good, 0.81–0.99 as very good, and 1 as perfect
339
340 138 agreement. If the contingency table reported one or more values equal to zero,
341
342 139 Cohen's kappa could not be calculated, thus in these cases the percentage of
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344 140 agreement was used. Baseline artifact duration was measured in ms, and expressed
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346 141 as median (range). Differences in the prevalence of baseline artifacts on smartphone
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348 142 and standard ECG tracings were evaluated using Fisher's exact test. Using the
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350 143 Bland-Altman test, bias and 95% limits of agreement were calculated for HR,
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363 144 duration of the P wave, PR interval, QRS complex and QT interval to analyze the
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365 145 differences between the smartphone and standard ECG.
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367 146 Statistical analyses were performed with commercial software (Microsoft Excel, 2011;
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369 147 GraphPad Prism 6, USA). A *P* value of <0.05 was considered significant.
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373 374 149 **Results**

375 376 150 *Animals and feasibility*

377
378 151 Thirty-two out of 50 horses were mares (64%), 15/50 (30%) were stallions, and 3/50
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380 152 (6%) were geldings, with a median age of 5 years (range: 2-29 years), median body
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382 153 weight of 474 kg (range: 300-652 Kg) and median BCS of 3/5 (range: 3-4/5). The
383
384 154 horses were of different breeds: 34/50 were Trotters (68%), 6/50 Thoroughbreds
385
386 155 (12%), and 10/50 Standardbreds (20%).

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388 156 On standard ECG tracings, all horses enrolled in this study showed sinus rhythm.

389 157 Among smartphone ECG tracings, 48/50 (96%) were judged acceptable for
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391 158 interpretation. The two smartphone ECG tracings considered inadequate for
392
393 159 interpretation showed baseline artifacts in most of the tracings, and were excluded
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395 160 from the analysis.
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399 162 *Heart rate*

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401 163 According to the standard ECG, 34/48 (71%) horses had a normal HR, 14/48 (29%)
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403 164 had tachycardia, and no horses showed bradycardia. A perfect agreement ($\kappa = 1$;
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405 165 95% CI: -1, 1) between the smartphone and standard ECG was found in the HR
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407 166 classification when it was manually measured on digitized tracings. The bias between
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409 167 the HR manually measured on standard ECG and smartphone ECG was 0.2 bpm
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411 168 (95% CI: -6.0, 6.4 bpm).
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423 169 The App HR was less accurate in detecting actual HR, with only a fair level of
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425 170 agreement compared to the standard ECG ($\kappa = 0.24$; 95% CI: -0.1, 0.4). The bias
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427 171 between App HR and HR manually measured on standard ECG tracings was -27.1
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429 172 bpm (95% CI: -105.1, 51.0 bpm). In most cases (22/48, 46%), the App HR
430
431 173 overestimated the HR measured on standard ECG.
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435 436 175 *P wave, PR interval, and QT interval duration*

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438 176 P wave duration measured on standard and smartphone ECG tracings showed a
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440 177 bias of 11.2 ms (CI 95%: -16.6, 39.1 ms), PR duration had a bias of 24.4 ms (CI
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442 178 95%: -18.4, 67.1 ms), and QT duration showed a bias of 0.8 ms (CI 95%: -54.0, 55.6
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444 179 ms). On the standard ECG tracings, the P polarity was positive in 31/48 (65%) cases
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446 180 and biphasic in 17/48 (35%). On the smartphone ECG tracings, the P polarity was
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448 181 positive in 12/48 (25%) and biphasic in 36/48 (75%) cases. No agreement was found
449
450 182 between the two methods in the P polarity evaluation ($\kappa = -0.05$; 95% CI -0.3, 0.1).
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454 455 184 *QRS complex analysis*

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457 185 The QRS complexes showed a negative polarity on standard ECG in all 48 analyzed
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459 186 cases (100%). On the smartphone ECG tracings, the QRS polarity was negative in
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461 187 46/48 (96%) cases and positive in 2/48 (4%). Hence, the resulting agreement
462
463 188 between the two methods in the QRS polarity evaluation was high (percentage of
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465 189 agreement: 96%). The QRS complex duration measured on standard and
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467 190 smartphone ECG tracings showed a bias of 6.5 ms (CI 95%: -23.4, 36.3 ms).
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471 472 192 *Artefacts*

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483 193 Of the 48 smartphone ECG tracings judged as interpretable, 11/48 (23%) presented
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485 194 baseline artifacts. The median duration of baseline artifacts on smartphone ECG
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487 195 tracings was 1480 ms (range: 960-3680 ms), corresponding to a median of 5% of the
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489 196 total duration of each ECG tracing. On the relative standard ECG tracings, only 1/48
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491 197 horse (2%) had baseline artifacts (duration: 560 ms). The presence of artifacts was
492
493 198 significantly higher in the smartphone ECG tracings than in standard ECG tracings
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495 199 (P=0.0037).
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500 201 **Discussion**

502 202 In our investigation, the smartphone ECG tracings were interpretable in 96% of
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504 203 cases. These results are in line with findings in humans where smartphone ECG
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506 204 tracings were interpretable in 87-99.6% of patients [8,9,17,24] and in dogs where
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508 205 traces were considered interpretable in 97.6% [22]. Only two smartphone ECG
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510 206 tracings were judged as non-interpretable due to the presence of too many baseline
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512 207 artefacts. In the other smartphone ECG tracings, 23% had short segments of
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514 208 baseline artefacts, compatible with motion or muscle tremor artifacts, however they
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516 209 did not hinder the assessment of mean HR and measurement of the
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518 210 electrocardiographic waves and intervals.

521 211 In our study, the smartphone ECG was reliable in measuring HR in horses, similar to
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523 212 findings in dogs, where a perfect agreement was found between smartphone and
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525 213 standard ECG in the evaluation of HR [22]. We observed the greatest accuracy when
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527 214 the HR was manually measured on digitized tracings. The accuracy of App HR was
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529 215 very poor in comparison to manually measured HR on digitized tracings.
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531 216 Furthermore, the App HR in horses proved less accurate than has been found in
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533 217 dogs [22]. In most smartphone ECG tracings, the App HR overestimated the real HR
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218 possibly because the App HR frequently interpreted tall T waves as QRS complexes.
219 In a few horses, the App HR was totally unreliable.
220 Regarding the evaluation of the ECG waves and interval durations, the smartphone
221 ECG was reliable, with minimal differences of no clinical value from the standard
222 ECG. A good agreement in the analysis of the QRS complex polarity was also found,
223 and in most horses, the QRS complexes showed the same polarity on smartphone
224 ECG and standard ECG tracings. This result is in line with a previous study in dogs,
225 in which the smartphone ECG showed a good agreement in the analysis of the QRS
226 complex, in assessing both duration and polarity [22].
227 No agreement in the analysis of P wave polarity was found between the two
228 methods. To the authors' knowledge, no previous studies in veterinary medicine have
229 been carried out on the agreement between standard ECG and smartphone ECG in
230 P polarity evaluation, thus our results cannot be compared with the literature.
231 Our investigation has some limitations. Firstly, no significant arrhythmias were
232 detected in this study, thus no statistical analysis was performed in order to assess
233 the diagnostic accuracy of the smartphone ECG in detecting cardiac arrhythmias.
234 However, this is the first study to evaluate the feasibility of the smartphone ECG in
235 recording good quality tracings in horses. Further studies are needed to evaluate the
236 diagnostic accuracy of smartphone ECG in detecting arrhythmias in horses.
237 Moreover, the smartphone tracings were acquired by only one operator and another
238 blinded operator judged whether or not they were acceptable for interpretation. Inter-
239 operator variability in the quality of ECG recording and interpretation was not
240 evaluated.

241
242 **Conclusions**

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602
603 243 The smartphone ECG can record single-lead ECG tracings of a good quality in
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605 244 **healthy** horses. It is reliable in the evaluation of HR and in the measurement of
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607 245 electrocardiographic waves and intervals. Smartphone ECG technology could
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609 246 represent a reliable additional tool in the electrocardiographic evaluation of horses,
610
611 247 however it is not a substitute for standard base-apex ECG. Further studies are
612
613 248 needed to assess the diagnostic value of the smartphone ECG in the diagnosis of
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615 249 arrhythmias in horses.
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626 254 **References**

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323 **Figure legends**

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325 Fig. 1. Dorso-ventral orientation with a 30° cranial inclination of the smartphone, with
326 the camera side of the smartphone located ventrally.

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328 Fig. 2. Smartphone ECG tracing showing sinus rhythm in a horse. Paper speed = 25
329 mm/s; 20 mm/mV.

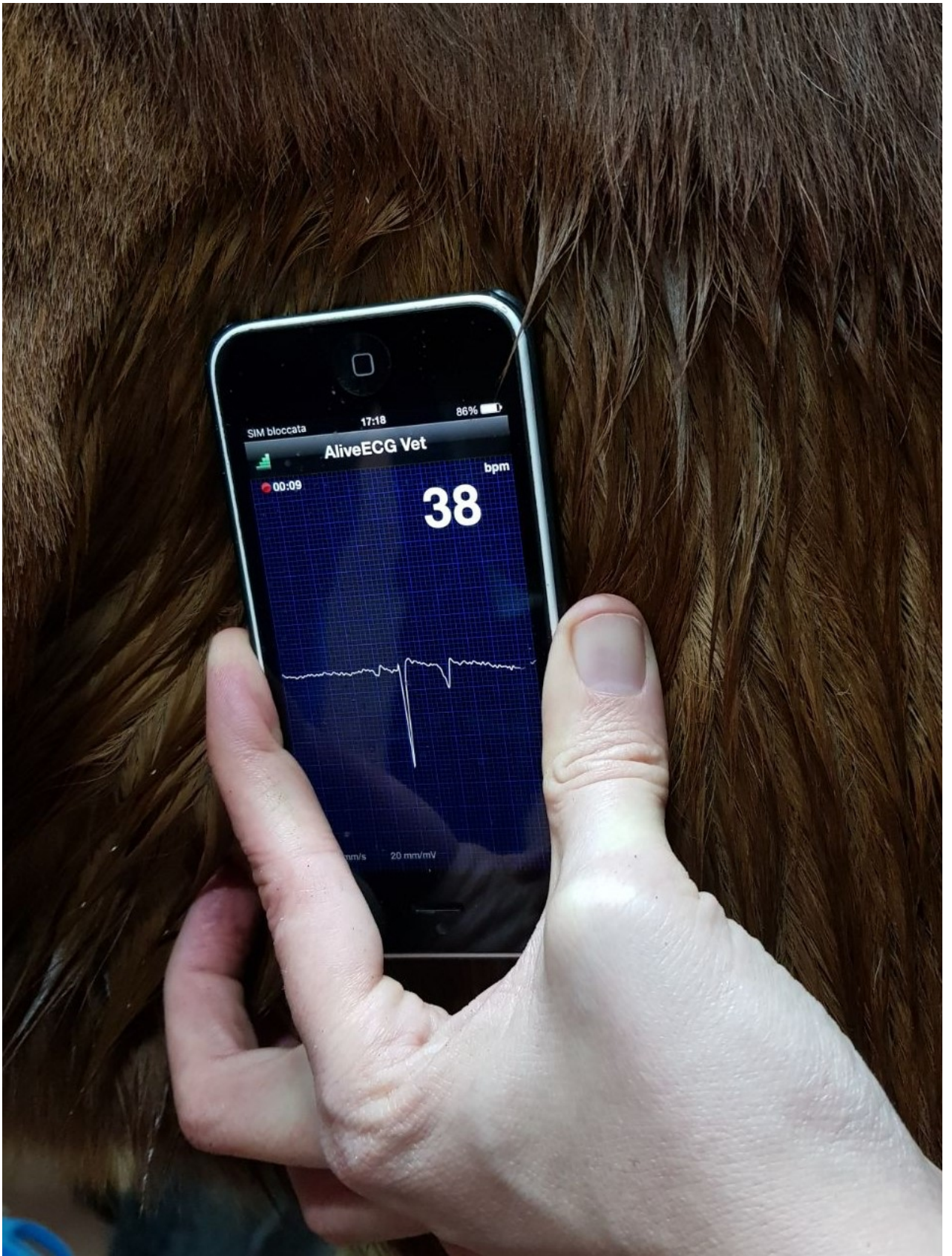
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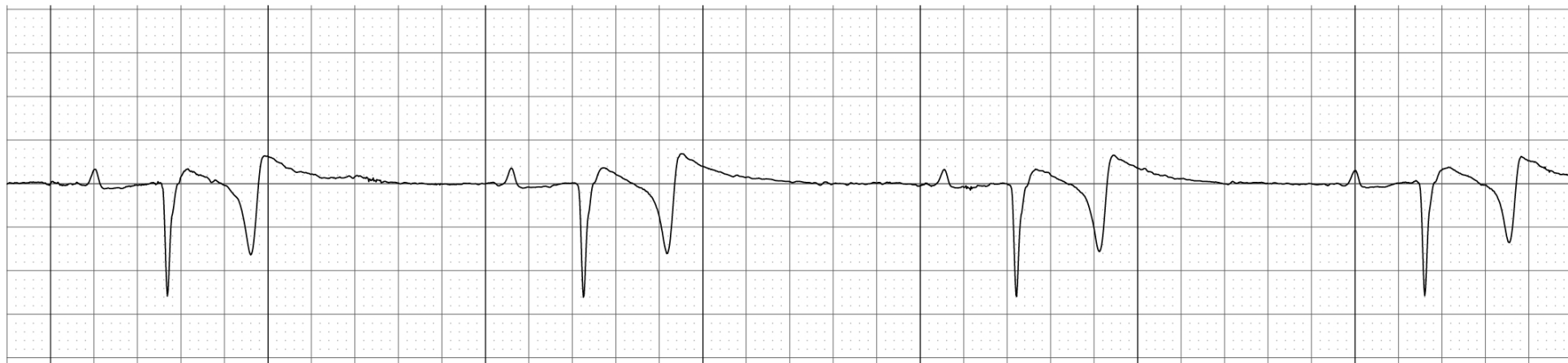
331 Fig. 3. Smartphone ECG tracing showing baseline artifacts in a horse. Paper speed =
332 25 mm/s; 20 mm/ mV.

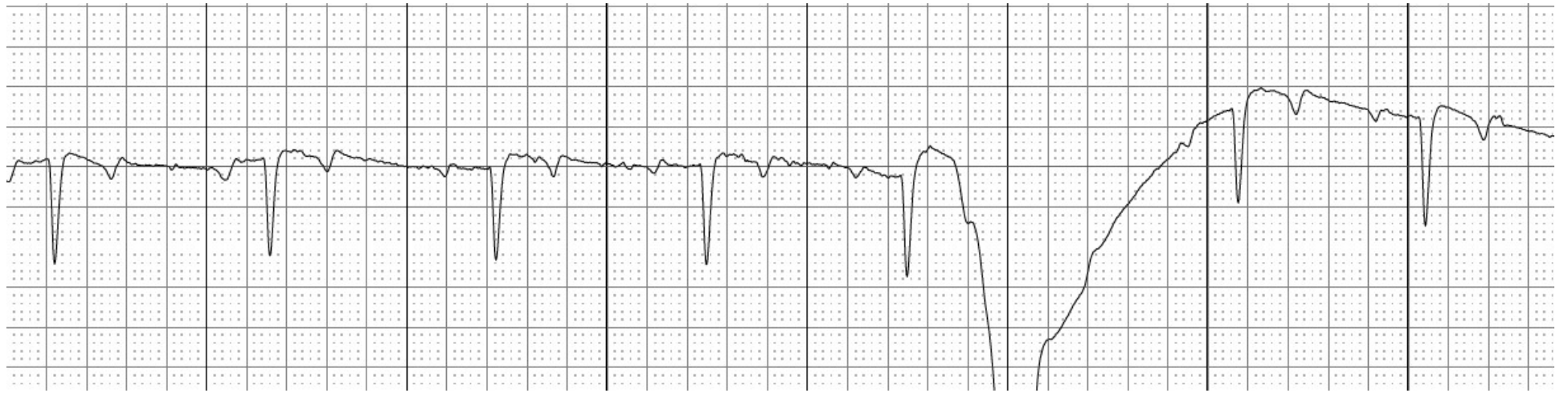
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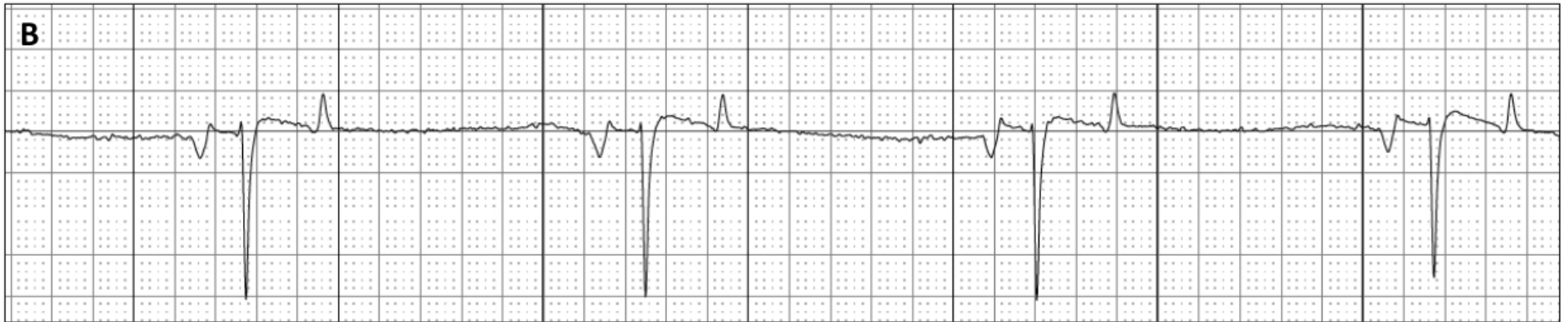
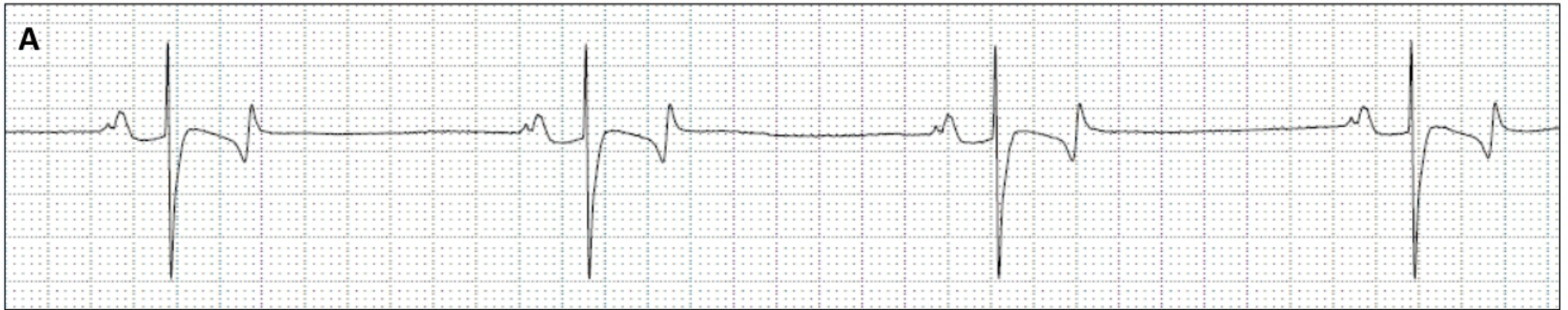
334 Fig. 4. Standard ECG (A) and smartphone ECG (B) tracings showing different P
335 wave polarity in a horse. Paper speed = 25 mm/s (A and B); 5 mm/mV (A) and 20
336 mm/mV (B).

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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. Tommaso Vezzosi

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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. 45965/2016). 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. Tommaso Vezzosi
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