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A novel approach for obtaining 12-lead electrocardiograms in horses

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Abstract

Background: In equine medicine, 12-lead electrocardiograms (ECGs) rarely are used, which may in part be a result of shortcomings in the existing guidelines for obtaining 12-lead ECGs in horses. The guidelines recommend placing the limb leads on the extremities, which is inappropriate because the ventricular mean electrical axis is then perpendicular to the limb leads, leading to large variations in ECG configuration even among healthy horses. From an electrophysiological point of view, the leads instead should be parallel to the electrical axis to minimize variability.

Objective: Develop an improved method for obtaining 12-lead ECGs in horses based on electrophysiology and cardiac electrical vectors relevant to horses.

Animals: Thirty-five healthy Standardbred horses.

Methods: Two ECGs obtained at rest; 1 ECG with the electrodes placed according to the method developed in the present study, the Copenhagen method, and 1 ECG following existing guidelines.

Results: In the Copenhagen method, we repositioned the limb electrodes to the thorax to better capture the electrical activity of the heart. Variation in the mean electrical axis decreased dramatically with the Copenhagen method (SD decreased from 24.6° to 1.6°, $P < .001$). Consequently, this new method provided stable ECGs with repeatable configurations.

Conclusions and Clinical Importance: With this novel method, the ECG is recorded with respect to the electric axis to fully realize the potential of 12-lead ECG in horses. The Copenhagen method delivered more consistent and reliable ECG recordings compared to existing guidelines. The Copenhagen method potentially allows for expanded use of 12-lead ECGs in equine medicine.

KEYWORDS

12-lead ECG, 12 lead, cardiology, Copenhagen method, ECG, equine, horse

Abbreviations: HR, heart rate; LA, left arm; LF, left foot; MEA, mean electrical axis; RA, right arm; RF, right foot; VCG, vectorcardiography; WCT, Wilson's central terminal.

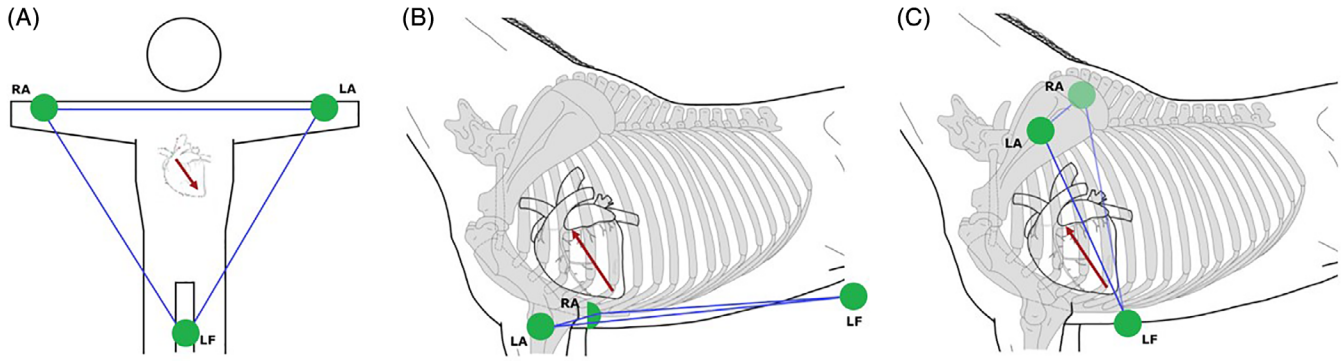

FIGURE 1 Einthoven's triangle and the placement of limb electrodes. A, Illustrates Einthoven's triangle in a human with the electrodes placed on the right (RA) and left (LA) arm and on the left foot (LF). The result is Einthoven's triangle around the heart, leads that follows the mean electrical axis (MEA, arrow) and Wilson's central terminal (WCT) in the middle of the heart. B, Illustrates the result of placing the electrodes on the limbs of a horse as described in the existing guidelines; Einthoven's triangle is perpendicular to the MEA (arrow). C, Illustrates the result of the Copenhagen method where the limb lead electrodes were moved to the thorax so Einthoven's triangle surrounds the heart and follows the MEA (arrow)

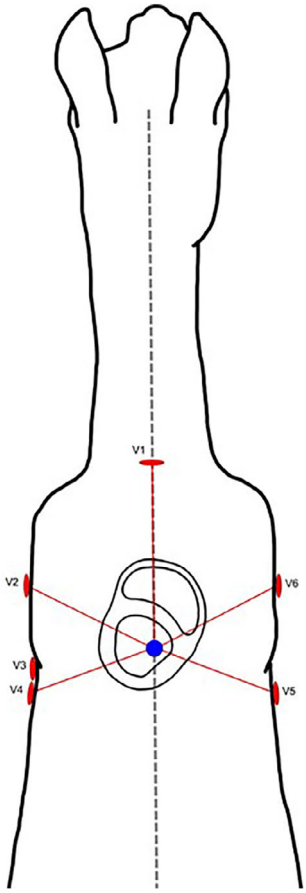

FIGURE 2 From the Copenhagen method, each of the precordial leads were measured from Wilson's central terminal (WCT, blue) to the electrodes placed on the horse (red). V1 approached the anterior wall of the RV, V2 the left part of the IVS, V3 the atria, V4 the left lateral wall of the LV, V5 the right lateral wall of the LV and V6 approached the right part of the IVS

TABLE 1 Electrode placement

Lead	Copenhagen method	Existing guidelines[11]
LA	On top of the left scapular spine	Just below the point of the elbow on the back of the left forearm
RA	On top of the right scapular spine	Just below the point of the elbow on the back of the right forearm
LF	Caudal to the xiphoid process, slightly left to the midline	On the loose skin at the left stifle in the region of the patella
RF	On top of the right scapular spine	On top of the right scapular spine
V1	Between the dorsal parts of the two descending superficial pectoral muscles	6th intercostal space on the left side of the thorax along a line parallel to the level of the point of the elbow
V2	Ventral part of the triceps muscle, left side	6th intercostal space on the left side of the thorax along a line parallel to the level of the point of the shoulder
V3	6th intercostal space, at level of the shoulder joint, left side	Over the dorsal thoracic spine of T7 at the withers
V4	6th intercostal space, at the level of the olecranon, left side	6th intercostal space on the right side of the thorax along a line parallel to the level of the point of the elbow
V5	6th intercostal space, at the level of the olecranon, right side	6th intercostal space on the right side of the thorax along a line parallel to the level of the point of the shoulder
V6	Ventral part of the triceps muscle, right side	On the top of the right scapular spine

1 | INTRODUCTION

The 12-lead electrocardiogram (ECG) is a valuable diagnostic tool in human cardiology to identify, not only electrical disorders such as atrial fibrillation and bundle branch block, but also hypertrophy and ischemia.[1,2] However, in equine medicine, ECGs are used solely for detection of rate and rhythm disturbances and only few leads are recorded. This limitation may, in part, be attributed to differences in physiology between horses and humans. In the horse, the Purkinje fibers run deep in the myocardium, as opposed to in humans and dogs, where the fibers run subendocardially, leading to a different ventricular activation pattern.[3,4] Furthermore, the orientation of the heart in the thorax differs among species. The human heart is nearly parallel to the spine, whereas the canine heart is more tilted in the thorax and the equine heart is almost perpendicular to the spine. Thus, the understanding from human cardiology cannot be directly translated to veterinary medicine, but studies have shown that multiple leads can be useful in differentiating arrhythmias in dogs and horses.[5-7] The ECG records the voltage difference between 2 electrodes and reflects the summation of the electrical activity of the heart. The net electrical activity has a magnitude and a direction and is thus by definition a vector. When the electrical vector's direction is parallel with the measured ECG lead, the deflection on the surface ECG will be maximal. Conversely, a vector that is perpendicular to the ECG lead will not give rise to any deflection. For that reason, the main electrical activity, characterized by the mean electrical axis (MEA), should preferentially be parallel to the recording leads.

In human cardiology, this requirement is fulfilled, because the MEA is directed downward and leftward and is captured using electrodes placed on the right arm (RA), left arm (LA), and left foot (LF),

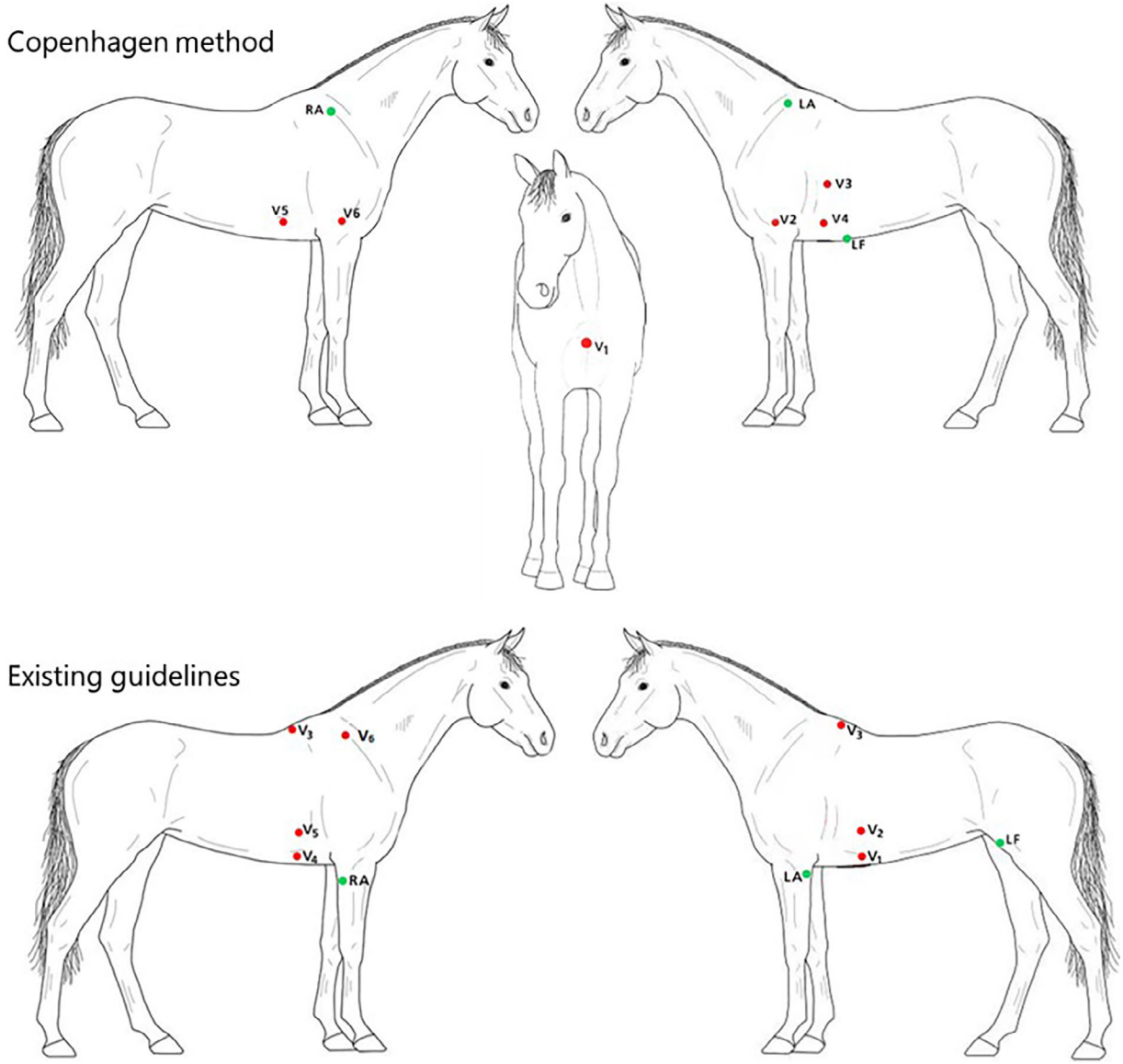

FIGURE 3 Electrode placement for the Copenhagen method (top panel) and a representation of the electrode placement of the existing guidelines (bottom panel)

giving rise to the limb leads (Figure 1A). In addition to the limb leads, 6 precordial leads (V1-V6) are placed on the chest to record electrical activity from the third spatial dimension. The combination of the limb leads and the precordial leads constitutes the 12-lead ECG.

The MEA of the horse heart points upward from the apex toward the base of the heart and, although no standardized method for electrode placement exists for horses, most equine clinicians place the electrodes for the standard 3-lead ECG in a similar way along the MEA, as a "modified base-apex" lead.[5,8-10] Few studies have explored the use of a 12-lead ECG in horses,[11-13] but at the time (1950-1980) the limb lead electrodes were placed on the legs. As a result, guidelines for obtaining a 12-lead ECG in horses today[14,15] still suggest placing the electrodes on the legs, which results in the MEA being almost perpendicular to the ECG leads (Figure 1B) leading to poor ECG quality.[6,16-26]

We aimed to develop an electrophysiologically sound method for obtaining 12-lead ECGs in horses in which the limb leads are parallel with the electrical activity of the heart. To evaluate the new method, we compared it to ECGs obtained using existing guidelines. We calculated the MEA and the variation and described ECG configurations, durations, and amplitudes for the 2 methods in healthy horses.

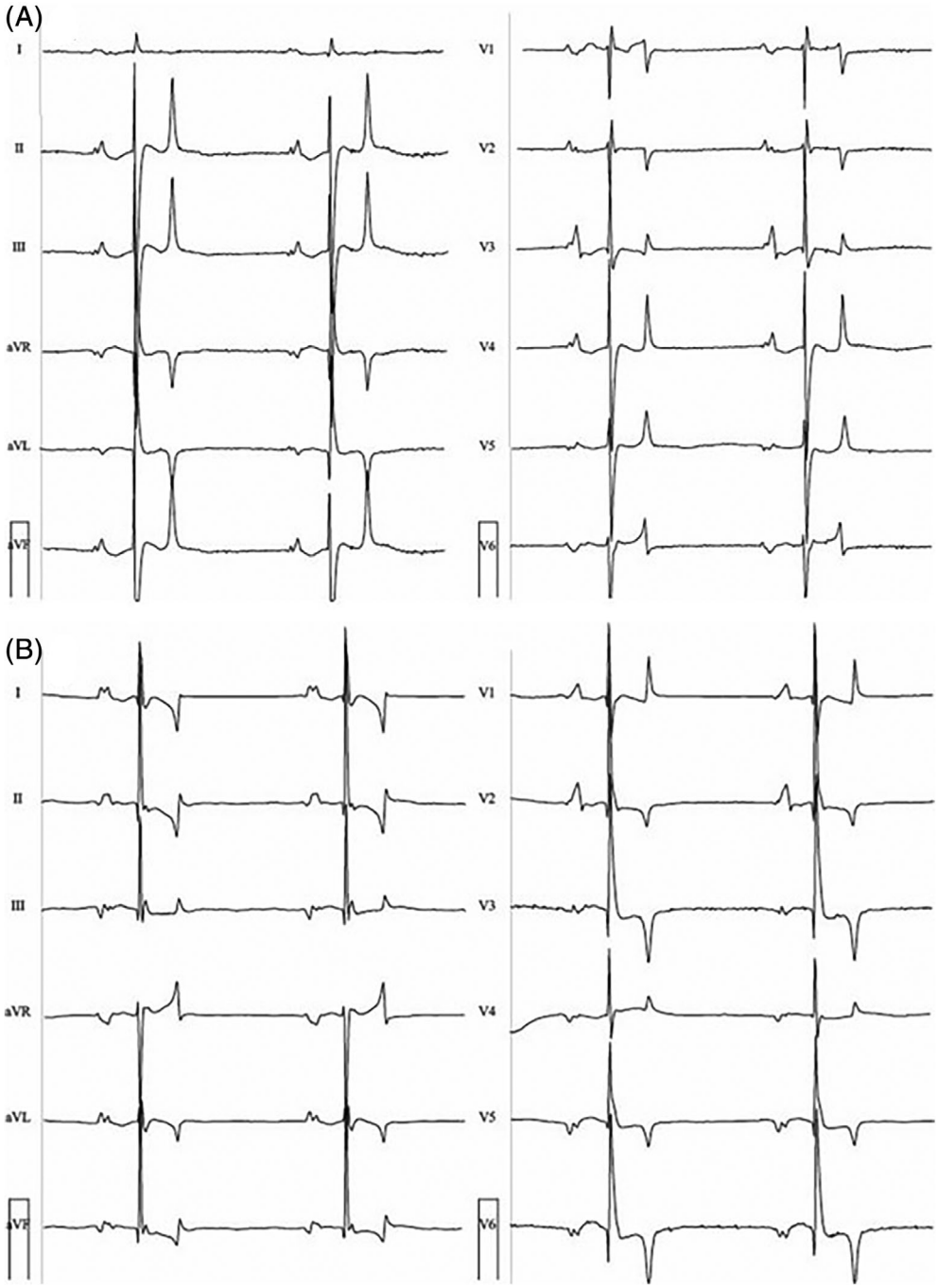

FIGURE 4 Example of a 12-lead ECG obtained with the Copenhagen method (A) and the existing guidelines (B) from the same horse. Paper speed 25 mm/s, gain 20 mm/mV

2 | MATERIALS AND METHODS

2.1 | Development of a new method for obtaining 12-lead ECGs

Investigation of the best electrode placement was performed on 1 horse using an analog 6-channel ECG recorder (SCHILLER AG, Baar, Switzerland) and paper speed of 25 mm/s.

The 3 limb leads (I, II, and III) form Einthoven's triangle and the average potential of these is termed Wilson's central terminal (WCT), which can be considered as an electrode placed in the middle of Einthoven's triangle. In the 12-lead ECG, WCT acts as the negative electrode for the precordial leads and therefore should be placed close to the heart.

Predefined criteria for electrode placement were as follows: the limb leads should be aligned with the MEA and span the vertical plane to have the heart and therefore WCT approximately in their center. Precordial leads preferably should be perpendicular to Einthoven's triangle, exhibit stable signal quality and cover the chambers of the heart in the horizontal plane.

The limb lead electrodes were positioned with the RA and LA electrodes placed on the top of the right and left scapular spine, respectively, and LF placed caudal to the xiphoid process, slightly left of the midline (Figure 1C). The reference ground electrode (RF) was placed next to the RA electrode. The electrodes for the precordial leads were placed side-by-side on the horse in horizontal rows from the descending superficial pectoral muscles to the tenth intercostal space at the level of the olecranon to the level of the shoulder. Several positions for the precordial electrodes resulted in good quality signals, and the final positions were chosen so each lead would record the heart's electrical activity from a different angle: V1 approached the anterior wall of the RV; V2, the left part of the interventricular septum; V3, the atria; V4, the left lateral wall of the LV; V5, the right lateral wall of the LV; and finally, V6 approached the right part of the interventricular septum (Figure 2). All precordial leads were placed in a horizontal plane at the approximate level of the ventricles except for V3. The V3 lead was placed at shoulder level to have a lead optimized for the atria and P wave analysis. The final electrode positions as well as the electrode positions for the existing guidelines are described in Table 1 and illustrated in Figure 3. This new method of 12-lead ECG is referred to as the Copenhagen method.

2.2 | Electrocardiographic examination

Thirty-five healthy Standardbred horses (14 mares, 15 geldings and 6 stallions) with an average age of 5.8 years (range, 2-14 years) and mean body weight (estimated by chest girth) of 503 kg (range, 431-588 kg) were included in the study. Seven horses were retired whereas the remaining 28 were in training. All horses had a clinical examination performed including body temperature, heart rate (HR), respiratory rate, and auscultation of the heart and lungs. In addition, a standardized echocardiographic examination was performed[27] to exclude cardiac diseases.

Two different ECGs were recorded using a digital GE 12-lead ECG system (GE Marquette CAM-14, GE Healthcare, Brøndby,

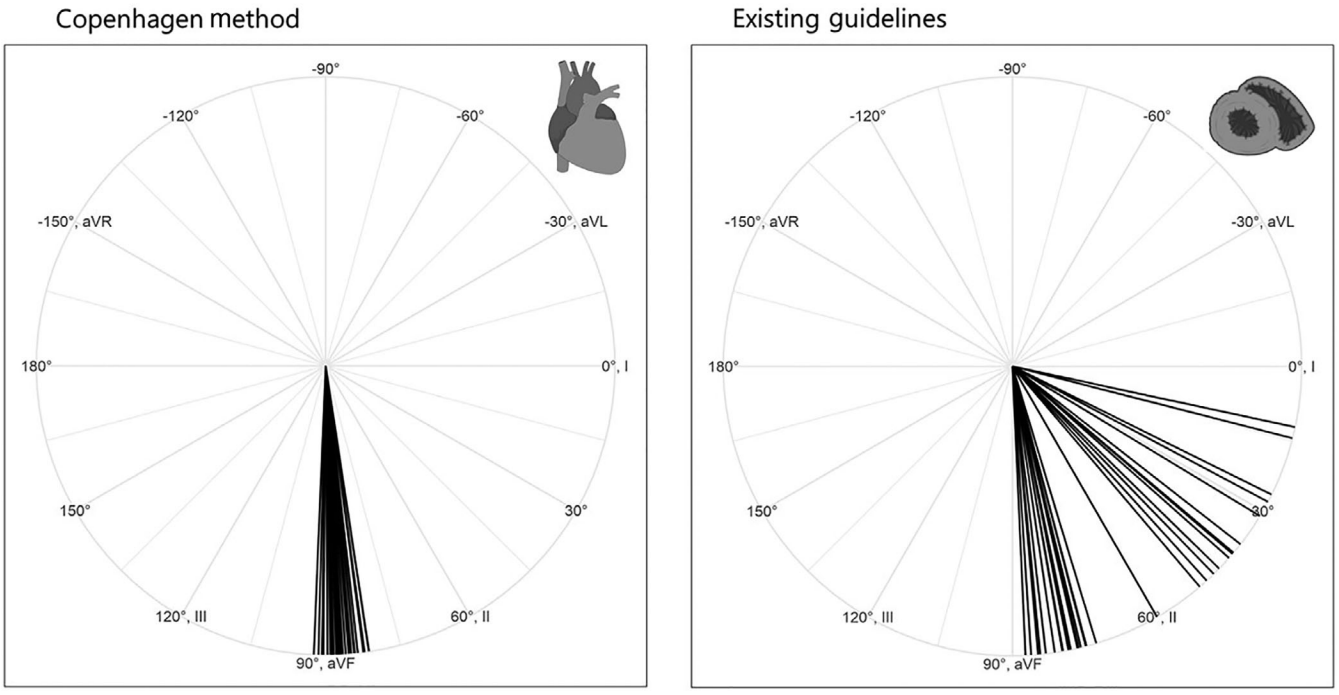

FIGURE 5 Distribution of the electrical axis of the P wave in 33 horses when the electrocardiograms (ECGs) was obtained with the Copenhagen method (left) and according to the existing guidelines (right). The variation was significantly less ($P < .0001$) in the Copenhagen method

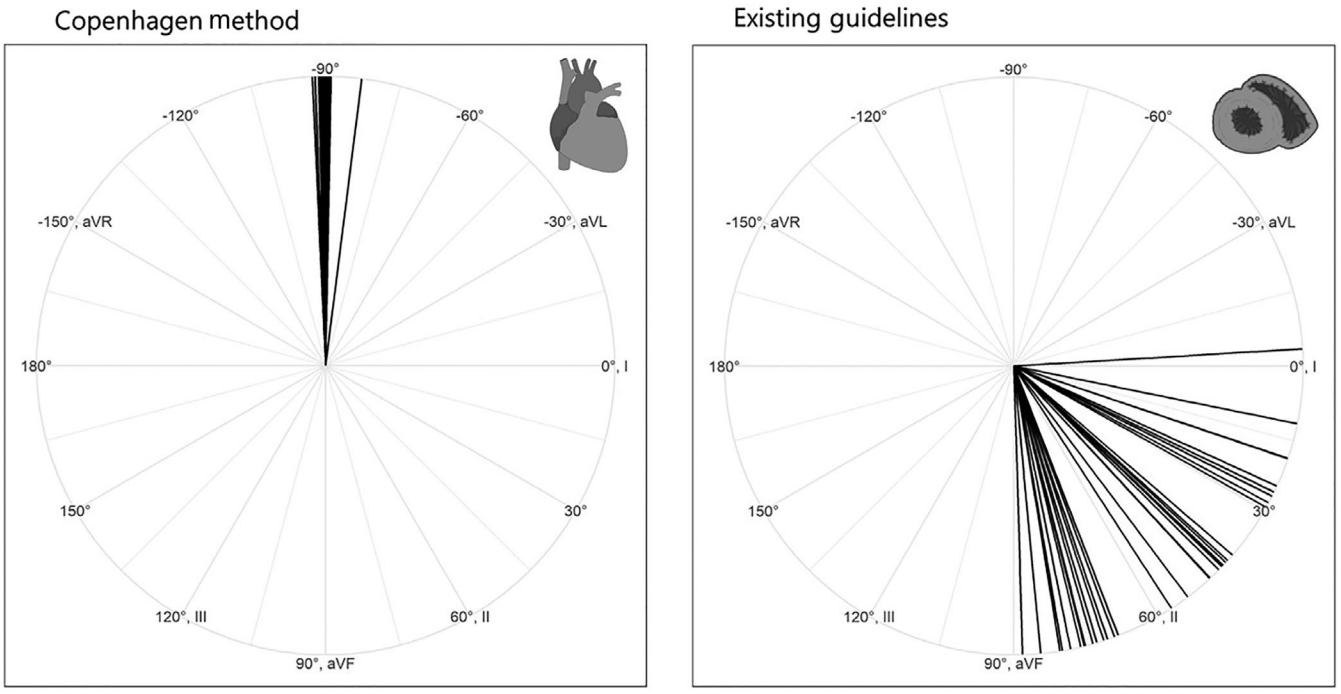

FIGURE 6 Mean electrical axis. Distribution of the mean electrical axis (MEA) in 33 horses when the electrocardiograms (ECGs) was obtained with the Copenhagen method (left) and according to the existing guidelines (right). The variation was significantly less ($P < .0001$) in the Copenhagen method

TABLE 2 Configuration of the P waves. The percentage distribution and their configuration of the P waves in all leads for both the Copenhagen method and the existing guidelines

Configurations of P waves expressed in %													
		I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
Copenhagen method	Bifid pos.	46	100	100			100		49	100	100	97	
	Bifid neg.				100	88		91	29			3	97
	Single peaked, pos												
	Single peaked, neg					6		3					
	Biphasic +/−					6		6	23				3
	Not recognizable	54											
Existing guidelines		I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
	Bifid, pos	94	82	67		27	73	82	79		58	21	
	Bifid, neg				88	30				97	6	42	100
	Single peaked, pos	3	9	18			9	12	15		24	3	
	Single peaked, neg				6	3				3	6		
	Biphasic +/−				6	33						6	
	Biphasic −/+	3	9	15			18	6	6		6	21	
	Not recognizable					6						6	

Denmark) and the electrodes (KRUUSE ECG Electrodes, Kruuse A/S, Maarslev, Denmark) were placed on unshaved skin. One ECG was recorded with the electrodes placed according to the Copenhagen method and another ECG was recorded according to the existing guidelines,[14] with the exception of V6 because it has not been described in the existing guidelines. Instead, a modified base-apex lead from the left side of the thorax to the right scapula is described,[14] but this additional lead cannot be recorded simultaneously using common ECG devices. In our study, V6 therefore was placed on the right scapular spine to best simulate a modified base-apex lead. The ECG device

TABLE 3 Amplitude and duration of all waves obtained by the Copenhagen method. Top panel: Mean amplitude ± SD of the P, Q, R, S, and T waves. Bottom panel: Mean duration ± SD of the P wave, the QRS complex and the PR and QT interval

Copenhagen method Absolute peak amplitude (mean ± SD) expressed in mV (n = 35)												
	I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
P	0.09 ± 0.02	0.32 ± 0.07	0.31 ± 0.07	0.19 ± 0.04	0.16 ± 0.04	0.31 ± 0.07	0.16 ± 0.04	0.11 ± 0.03	0.44 ± 0.07	0.31 ± 0.07	0.16 ± 0.04	0.16 ± 0.03
Q	0.05 ± 0.03	…	…	0.23 ± 0.13	0.23 ± 0.12	…	0.23 ± 0.13	0.20 ± 0.14	0.06 ± 0.02	0.06 ± 0.02	…	…
R	0.16 ± 0.05	0.46 ± 0.25	0.45 ± 0.25	0.89 ± 0.17	1.09 ± 0.16	0.48 ± 0.25	0.27 ± 0.15	0.18 ± 0.13	0.43 ± 0.23	0.51 ± 0.30	0.28 ± 0.13	0.10 ± 0.05
S	…	1.93 ± 0.31	2.07 ± 0.32	…	…	2.01 ± 0.32	0.27 ± 0.17	0.29 ± 0.19	0.58 ± 0.40	1.66 ± 0.48	1.11 ± 0.22	0.74 ± 0.24
T	0.09 ± 0.04	0.76 ± 0.19	0.73 ± 0.19	0.39 ± 0.10	0.37 ± 0.09	0.75 ± 0.19	0.34 ± 0.10	0.23 ± 0.10	0.43 ± 0.15	0.79 ± 0.32	0.41 ± 0.17	0.42 ± 0.18
Durations and intervals (mean ± SD) expressed in ms (n = 35)												
	I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
P	139 ± 18	158 ± 19	157 ± 19	157 ± 18	148 ± 20	156 ± 19	137 ± 15	131 ± 14	146 ± 16	152 ± 17	153 ± 16	139 ± 16
QRS	75 ± 19	120 ± 12	119 ± 13	114 ± 11	116 ± 10	119 ± 11	110 ± 19	120 ± 16	117 ± 16	117 ± 12	116 ± 9	113 ± 17
PR	389 ± 51	376 ± 58	372 ± 57	375 ± 56	361 ± 53	374 ± 56	357 ± 54	368 ± 62	376 ± 54	373 ± 56	373 ± 55	367 ± 57
QT	461 ± 28	513 ± 25	511 ± 25	504 ± 24	502 ± 23	510 ± 24	503 ± 28	488 ± 26	504 ± 26	509 ± 23	500 ± 20	508 ± 34

was secured to the horse using an elastic girth. Five-minute ECG recordings were obtained at rest with HR <45 bpm.

2.3 | Data analysis

The ECGs were analyzed manually using the software of the ECG recorder (Cardiosoft, GE Healthcare) on 3 consecutive heartbeats with the observer blinded to the method used. The durations of the P wave and QRS complex were measured and amplitude and morphology of the P, Q, R, and S waves were measured and described. The P wave was described as follows: a single-peaked P wave was described according to the direction (positive or negative), a 2-peaked P wave with the same direction was denoted as "bifid" and a 2-peaked P wave with opposite directions was denoted "biphasic." The QRS complex was described as follows: all positive upstrokes after the P wave were denoted "R," all negative downstrokes preceding an R wave were denoted "Q" and all negative downstrokes after an R wave were denoted "S." In addition, for the Copenhagen method, the PR and QT intervals were measured and T wave morphology was described for future reference.

The electrical axes for the P wave and MEA were calculated for both methods by trigonometric calculation.[28] We used the largest amplitude (regardless of orientation) of the P wave and the QRS complex from leads II and aVL because they consisted of large complexes, which allowed for more accurate measurement of the amplitudes. The equation used was based on the trigonometric tangent and aligned so that 0° corresponds to lead I:

$$\text{Electrical axis} = \arctan\left(\frac{\text{Amp}_{\text{II}}}{\text{Amp}_{\text{aVL}}}\right) - 30^{\circ}$$

where Amp is the largest amplitude of the wave of interest. An *F* test of equal variance was used to compare the variance of the electrical axes for the 2 methods. Comparisons of waves between 2 leads were performed using a paired *t* test. The consistency in morphology in each lead was described by use of entropy (*S*), defined by:

$$S = -\sum_{i=1}^{\text{no of beat classes}} p_i \times \ln(p_i)$$

where p_i is the proportion of beats belonging to class *i*. The entropy describes the distribution of morphologies. If all horses had had the same morphology, the entropy would be 0 and if they all fell in different groups the entropy would be 2.5 for the QRS complexes and 1.9 for the P waves; the higher the entropy, the more variation.

We used the nonparametric Mann-Whitney test to compare entropy between the methods. Data tested with a parametric test are shown as mean ± SD and data tested with a nonparametric test are presented as median (interquartile range [IQR]). For all statistical analyses, $P < .05$ was considered significant.

3 | RESULTS

An ECG example is presented in Figure 4, where ECGs from both methods were obtained from the same horse. Two ECGs obtained using the existing guidelines were excluded because of recording errors.

3.1 | Electrical axes

The average electrical axis of the P wave was 86.6° ± 2.8° (range, 81.4°-92.4°) in the Copenhagen method and 61° ± 23° (range, 12.1°-87.4°) using the existing guidelines (Figure 5). The variation of the electrical axes was significantly lower in the Copenhagen method ($P < .0001$).

Using the Copenhagen method, the average QRS MEA was −90.2° ± 1.6° (range, −92.6° to −82.9°) whereas it was 51.9° ± 24.6° (range, −3.4° to 88.3°) using the existing guidelines. The distribution of the MEA is shown in Figure 6. Variation in the MEA was significantly lower in the Copenhagen method compared to the existing guidelines ($P < .0001$).

3.2 | Morphology of the P wave and QRS complex

The P waves measured using the Copenhagen method generally had a bifid configuration with a positive upstroke in the majority of the leads (leads I, II, III, aVF, V2, V3, V4, and V5). In leads II, III, aVR, aVF, V1, V3, V4, V5, and V6, this configuration was consistent (>90% bifid configuration; Table 2). The median entropy of the P wave configuration using the Copenhagen method was 0.06 (IQR, 0.0-0.4). The amplitude of the P waves varied among leads with the largest amplitude in V3 and the lowest in lead I (Table 3). The V3 electrode was placed higher on the thorax than the other precordial leads in order to have a lead optimized for P wave analysis. Comparison of P wave amplitudes between V3 and V4 in the Copenhagen method indicated a significantly higher P wave amplitude ($P < .0001$) in V3.

With the existing guidelines, the P waves varied in configuration with and among leads (Table 2) with an entropy of 0.6 (IQR, 0.3-1.1) which was significantly higher than that of the Copenhagen method ($P < .01$). Lead V6 showed only bifid configuration and this configuration also was dominant in the majority of the limb leads (67%-94%). The configuration of the P waves varied most in leads aVL, V4, and V5, with some P waves unrecognizable in aVL and V5. The highest P wave amplitude was observed in lead V2. The V2 electrode using the existing guideline was positioned identical to V3 in the Copenhagen method, but the reference electrode differed between the methods because the position of WCT differed. The P wave in lead V3 using the Copenhagen method had a significantly longer duration (Copenhagen method, 146 ± 16 ms; existing guidelines, 123 ± 22; $P < .0001$) and higher amplitude (Copenhagen method,

TABLE 4 Configuration of the QRS complex. The percentage distribution and their configuration of the QRS complex in all leads for both methods

Configurations of QRS complex's expressed in %													
		I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
Copenhagen method	QRS							14	11	31	3		
	QR	6			100	100		9	3				
	RS		100	100			100	14	51	66	97	100	97
	R	94						3					
	QS								6	3			3
	RRS							3	6				
	RSR							37	6				
	RSRS							20	17				
Existing guidelines		I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
	QRS	61	18	12		12	18	70	39		21		
	QR	12	42	36	6		36		45	100	6	67	100
	RS	21	3		55	45	3	27	6		42	3	
	R	3				3						3	
	QS				3	6		3	3				
	QRSR		9	36			24		3		12	12	
	QRR		27	12			18		3			6	
	QRRS	3		3									
	RR											9	
	RRS					3							
	RSR				36	9					18		
	RSRS					21							

TABLE 5 Amplitude and duration of all waves measured from electrocardiograms (ECGs) obtained by the existing guidelines. Top panel: Mean amplitude and SD of the P, Q, R, and S waves. Bottom panel: Mean duration and SD of the P wave and the QRS complex

Existing guidelines												
Absolute peak amplitude (mean ± SD) expressed in mV (n = 33)												
	I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
P	0.13 ± 0.04	0.26 ± 0.06	0.19 ± 0.01	0.18 ± 0.03	0.01 ± 0.03	0.21 ± 0.06	0.24 ± 0.06	0.36 ± 0.09	0.12 ± 0.04	0.11 ± 0.04	0.09 ± 0.04	0.20 ± 0.03
Q	0.06 ± 0.03	0.16 ± 0.08	0.17 ± 0.13	0.60 ± 0.53	0.29 ± 0.43	0.14 ± 0.07	0.07 ± 0.03	0.14 ± 0.08	0.21 ± 0.09	0.06 ± 0.10	0.06 ± 0.03	0.23 ± 0.11
R	0.58 ± 0.26	1.14 ± 0.51	0.71 ± 0.47	0.11 ± 0.04	0.26 ± 0.20	0.92 ± 0.47	0.62 ± 0.35	0.91 ± 0.41	1.39 ± 0.23	0.44 ± 0.23	0.63 ± 0.25	1.25 ± 0.23
S	0.10 ± 0.06	0.13 ± 0.07	0.25 ± 0.17	0.85 ± 0.39	0.22 ± 0.14	0.14 ± 0.09	0.77 ± 0.45	0.14 ± 0.12	…	0.32 ± 0.18	0.15 ± 0.13	…
Duration and intervals (mean ± SD) expressed in ms (n = 33)												
	I	II	III	aVR	aVL	aVF	V1	V2	V3	V4	V5	V6
P	130 ± 21	134 ± 22	124 ± 21	137 ± 20	123 ± 18	130 ± 23	133 ± 22	123 ± 22	161 ± 23	121 ± 25	129 ± 26	153 ± 17
QRS	117 ± 19	116 ± 18	121 ± 21	97 ± 19	108 ± 18	121 ± 17	106 ± 13	111 ± 16	126 ± 9	104 ± 16	113 ± 17	124 ± 8

0.44 ± 0.07 mV; existing guidelines, 0.36 ± 0.09 mV; $P < .0001$) than the P wave obtained from the same electrode (V2) using the existing guidelines.

Using the Copenhagen method, the dominant configuration of the ventricular depolarization was the RS (II, III, aVF, V4, V5, and V6) and QR (aVR and aVL) with 100% consistency in the limb and augmented leads (except for lead I where only an R wave often was recognizable). In lead V3, 31% of the horses displayed a QRS configuration whereas lead V1 and V2 showed the greatest variation of QRS morphology (Table 4). The median entropy of the QRS complexes was 0.06 (IQR, 0.0-0.6). The duration of the QRS complexes was consistent in all leads except for lead I where the average QRS duration was 40 ms shorter (Table 3).

The configuration of the QRS complexes obtained using the existing guidelines showed much variation in all leads except for leads V3 and V6, where all horses showed a QR configuration. This resulted in a median entropy of 1.17 (IQR, 0.8-1.4) which was significantly higher than the entropy using the Copenhagen method ($P < .05$). Leads I, II, III, aVF, V2, V4, and V5 showed >5 different configurations and up to 7 in lead aVL (Table 4). The duration of the QRS complex varied among the leads, with aVR having the shortest duration and V3 the longest (Table 5).

4 | DISCUSSION

A main requirement of obtaining quality ECGs is to minimize recording variation in normal beats, so that abnormal beats can be recognized. We established a novel method for obtaining 12-lead ECG in horses with stable configurations, small variation, and leads that covered the entire heart. We achieved these results by relocating the "limb leads" from the limbs to the thorax. Doing so has 2 clear advantages. First, lead II is now parallel to the MEA, which decreased variation of the MEA significantly ($P < .0001$) compared to existing guidelines. Here, the electrodes were placed on the extremities of the horse perpendicular to the MEA. This approach previously has been addressed and criticized in another study.[18] The decreased variation of the MEA in the Copenhagen method was evident on the ECG because the QRS configuration in all limb leads was consistent. In addition, lead II reflects the modified base-apex ECG, providing the clinicians with a familiar lead in addition to the precordial leads. However, repositioning of Einthoven's triangle resulted in a shorter distance between the RA and LA electrodes and an almost perpendicular angle of the MEA to lead I, resulting in small deflections in lead I. In healthy horses, this lead therefore will be less useful but potentially could be important for diagnosing cardiac conditions.

The second advantage to repositioning Einthoven's triangle is that WCT is located near the heart and not displaced to the abdomen. Using the Copenhagen method, WCT is slightly dorsal to the heart because electrode placement was chosen in order to have good anatomical markers and as little muscle artifact as possible. The MEA is close to parallel with the recording plane (Einthoven's triangle) leading to good ECG recordings. This outcome was clearly evident in the P wave analysis, where we found a significantly ($P < .0001$) larger

amplitude and longer duration of the P wave in the V3 lead in the Copenhagen method compared to lead V2 using the existing guidelines although the electrodes for these 2 leads are positioned identically. In other words, repositioning of the WCT reference electrode gives better ECGs in the precordial leads.

Using the Copenhagen method, we found that the configuration of the P wave was significantly ($P < .01$) more consistent compared to the existing guidelines. The bifid configuration was the dominant configuration in accordance with a depolarizing wave front originating from the sinus node because the bifid configuration reflects first the depolarization of the right atrium and subsequently the depolarization of the left atrium.[25] A previous study[16] found the P vector to point caudal, ventrally and slightly to the left in most horses, and a similar result was found in another study[23] recording from both horizontal and transverse planes. These results corresponded with our observations because leads II, III, V3, and V4 record in a similar direction, and these leads had the largest P wave amplitude with consistent and well-defined configuration. On the contrary, leads V2, V5, and aVL recorded at a greater angle to the dominant P vector, and these leads had low amplitudes and generally were difficult to measure.

In the horse, the first part of the ventricular depolarization visible on the ECG occurs in the endocardial part of the left septum directed toward the apex.[3,21] This first depolarization results in an R wave in leads II, III, and aVF and a Q wave in leads aVR and aVL when the leads are placed along the MEA. The second part of the depolarization, which is often the largest (the MEA), is directed from the apex toward the base, resulting in an S wave in leads II, III, and aVF, but an R wave in leads aVR and aVL. These previously reported findings were in accordance with our results because leads II, III, and aVF had an RS configuration and leads aVR and aVL had a QR configuration.

One study[3] described the vector to be upward and slightly right whereas other studies[2,3,19] showed the vector to be upward and slightly to the left,[19] using both semiorthogonal leads (vectorcardiography [VCG]) and Einthoven's triangle in the transverse plane (similar to the placement of Einthoven's triangle using our method). Our findings support an upward and slightly leftward vector because the MEA ranged from −92° to −82° and no S waves were present in lead I. A recent study[28] investigated the positions of the limb lead electrodes on horses to optimize recording of the MEA and found the smallest variation in the MEA when placing the limb lead electrodes in a similar manner as in our method. This study also suggested the MEA to be slightly leftward (−79.9° ± 7.4°).[28]

For the ECGs obtained using existing guidelines, the configuration of the QRS complex in the limb leads varied substantially and the direction of the MEA ranged from −3° to +88°. This observation is in accordance with previous VCG studies that placed Einthoven's triangle in the horizontal plane (electrodes on the legs) and where the QRS vector in 377 horses varied from −70° to +110°.[16] The large variations when Einthoven's triangle is placed underneath the horse is the result of an almost perpendicular angle between the MEA and the recording leads, as opposed to the small variation when the recording leads are parallel to the MEA. Our observations further confirm that Einthoven's triangle is not useful when obtained from the limbs of horses, but corresponds very well when obtained from the thorax as when using our method. In our study, the configuration of the QRS complex also varied in the precordial leads obtained using the existing guidelines, consistent with data from previous studies.[11-13] This finding was further supported by the entropy calculations, which showed significantly ($P < .05$) more consistent morphologies using the Copenhagen method compared to the existing guidelines.

Of the precordial leads in the Copenhagen method, V1 and V2 were most perpendicular to the MEA and these leads also showed the smallest amplitude and largest variation in configurations. As recorded by leads V3 and V4, the angle to the MEA became more parallel resulting in both large R and S waves and a constant configuration. As recorded by leads V5 and V6, the angle became steeper again and the amplitude decreased in both leads. This finding corresponds well with previous studies of the QRS vector in the transverse plane.[19,23]

In the equine clinic 1 to 3 leads are used, which is sufficient for simple arrhythmia diagnosis. Multiple leads however previously have proven useful in the detection and evaluation of premature beats.[5] Using a 12-lead ECG with electrodes placed correctly, the entire heart is covered in a 3-dimensional space, and premature beats (or other conduction abnormalities that change the vector) not visible on the regular ECG may become detectable. Preliminary data suggest that the origin of ventricular ectopic beats can be predicted with up to 93% accuracy using the Copenhagen method.[29] To recognize changes as pathological, it is important to be able to record ECGs that in healthy horses provide stable configurations, which we achieved by developing the Copenhagen method. Future studies should include horses with cardiac diseases to test this hypothesis.

5 | LIMITATIONS

The 12-lead ECG has many cables and electrodes, which can be challenging when dealing with young or restless horses, but also limits applicability to resting ECG. Therefore, a modified base-apex, where the 3 electrodes can be secured beneath an elastic girth still is required for the nervous horse or when performing exercise tests. Two of the precordial leads (V2 and V6) were placed on the triceps, and these leads might be more vulnerable to limb movement. The 1/2/3 leads ECG also still is preferred when the clinician suspects a simple arrhythmia, whereas the 12-lead ECG might be useful for more complex cases or to determine the origin of the arrhythmia. There are limitations however to the ECG in general and despite less variation in the Copenhagen method, it still may not fully capture all equine cardiac activation patterns. Our study only included healthy horses and the Copenhagen method should be applied to horses with cardiac diseases in future studies to explore its full potential. Furthermore, other breeds should be studied to elucidate potential breed differences.

6 | CONCLUSION

We have designed a novel method for obtaining 12-lead ECGs specifically for horses, where the limb leads were positioned with respect to

the equine cardiac vectors. The superiority of this new method was evident by the significant ($P < .0001$) decrease in variation in the MEA. The Copenhagen method provides high quality ECG recordings with consistent configuration of the P waves and the QRS complexes. The Copenhagen method may help clinicians in detecting the origin of extrasystoles and other diseases in which a change in electrical activation will be visible on the 12-lead ECG. To test the translatability of the method from healthy to diseased horses the method should be applied to horses with cardiac arrhythmias.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Local ethic committee approval and owner informed consent was obtained prior to the study. Approval from the Danish Animal Experiments Inspectorate was not required as this was a non-invasive study.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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