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Factors affecting the mean electrical axis of the heart in trained Thoroughbreds

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Summary

Background: Assuming that the ventricular myocardium of horses is subjected to exercise-induced hypertrophy, we hypothesised that the mean electrical axis (MEA) of the heart would change.

Objectives: To define a longitudinal study to detect any changes in the direction of the MEA in Thoroughbred horses using ECG.

Study design: ECGs were recorded on each horse in each training group at day 0 (T0), 1 month (T1) and 2 months (T2) of training.

Methods: A total of 43 Thoroughbred horses in training in Italy were recruited. The horses were divided into three groups according to age. The ECGs were recorded by positioning the electrodes according to Dubois's method for measuring MEA in the frontal plane. Intervals with artefact-free QRS complexes in both bipolar DI and augmented unipolar aVF leads were selected, and the vector obtained was identified as the MEA. The statistical analysis was performed via generalised linear mixed model (GLMM) and principal component analysis (PCA).

Results: A statistically significant effect of time passing between T0 and T2 ($p < 0.001$) and an interaction between time and sex on the MEA was found ($p = 0.04$). PCA revealed that the population studied had different patterns, with three horses showing higher variability in the MEA direction.

Main limitations: There was no good sex balance in the age groups of the population studied, and there was no control group. The 1-month sampling intervals of ECGs may have been too short. Confirmatory studies are needed.

Conclusions: We believe that our results are the first to suggest that training may lead to changes in MEA orientation in horses. Sex and individuality were found to influence MEA orientation and may have contributed to the difficulty in detecting training-dependent changes in MEA to date.

KEYWORDS

horse, ECG, electrodes, frontal plane, MEA, training

Martina Felici and Paola Pratelli should be considered joint first authors.

INTRODUCTION

When a cardiac cell depolarises or repolarises, currents flow across the cell membrane creating an action potential and an electrical field at the body surface. When more cardiac cells depolarise or repolarise simultaneously, this electrical field can be detected and recorded by an ECG using electrodes placed on the skin. The mean electrical axis (MEA) represents the sum of the action potentials of all individual cardiac cells and indicates the "mean" direction of the cardiac electrical activity on the frontal plane (van Loon & Patteson, 2010; Verheyen et al., 2010). The MEA is a vector defined by magnitude and direction (da Costa et al., 2017). The magnitude of the MEA depends on the number of myocardial fibres affected by the electrical phenomenon, while the direction depends on both the depolarisation site of origin and its diffusion (Porciello, 2003). Although it is possible to calculate the MEA of individual ECG waves, it is more common to measure it at the level of the QRS complex, which indicates ventricular depolarisation (Kuhn & Rose, 2008).

Under physiological conditions and given that the left ventricular myocardium is larger than the right ventricular myocardium in humans and small animals, the MEA of the QRS is directed downwards and to the left (Brojbă, 2018; Marr & Bowen, 2010). The MEA can change from this physiological direction because of system changes in functionality or myocardial cells being damaged (Kuhn & Rose, 2008). In fact, in human and small animal medicine, the MEA is used in clinical diagnostics to assess defects in conduction and changes in the size of cardiac chambers (Kuhn & Rose, 2008). However, also due to the different morphology of the myocardium, the MEA has long been taught to have less diagnostic importance in horses (Marr & Bowen, 2010). However, it appears that the significance of the information obtained from the horse's MEA also depends on the correct positioning of the electrodes, as opposed to the old positions with the leads on the horse's limbs (Van Steenkiste et al., 2022).

Purkinje fibres terminate at the sub-endocardial level in humans and small animals, and depolarisation proceeds to the subepicardial layers through the common myocardium (Gómez-Torres et al., 2021; Hamlin & Smith, 1965). In these species, cardiac hypertrophy can increase the amplitude and duration of the QRS complex as depolarisation takes longer to diffuse into the ventricular myocardium (Tilley, 1985).

In horses, Purkinje fibres extend into the thickness of the ventricle wall, and depolarisation is transmitted to the common myocardium at multiple sites simultaneously (Gómez-Torres et al., 2021; Hamlin & Smith, 1965). The electrical forces that are generated tend to cancel each other, and the overall effect of ventricular myocardial depolarisation on ECG tracing is minimal (Marr & Bowen, 2010). Therefore, in horses, the duration of the QRS complex does not seem to indicate changes in ventricular size (van Loon & Patteson, 2010).

As in humans, the ventricular myocardium in horses is subject to exercise-induced hypertrophy (Marr & Bowen, 2010). Post-mortem findings have reported that the cardiac mass of athletic horses was higher than in untrained subjects (Kubo et al., 1974; Marr & Bowen, 2010), and the use of echocardiography confirmed this evidence (Evans & Young, 2010; Young, 1999; Young et al., 2005).

However, it is difficult to standardise echocardiographic findings in longitudinal comparisons between horses in training (Young et al., 2005). Moreover, changes in echocardiographic parameters (i.e. increased left ventricular size) have been recently shown to be associated with changes in some ECG parameters in trained horses, compared with sedentary subjects (Chanda & Petchdee, 2022; Cherdchutham et al., 2020). These data suggest that the ECG may also detect the morpho-functional changes that the heart undergoes as a result of physical activity in the horse. The horse is a model athlete for the study of cardiac response to training (Chanda & Petchdee, 2022). As a result of cardiac adaptation to exercise, the equine heart optimises oxygen transport and stroke volume (Chanda & Petchdee, 2022). Investigating whether and how exercise can modify the morphology and physiology of the heart is therefore of interest not only in veterinary medicine but also in sports medicine in general.

In human athletes and laboratory animals, myocardial hypertrophy may increase the time it takes for the wavefront to propagate throughout the myocardium, thus modifying the MEA (Drezner et al., 2017; Gray & Semsarian, 2020; Marr & Bowen, 2010).

Assuming that the horse's ventricular myocardium can be subject to exercise-induced cardiac hypertrophy, we hypothesised that there would be changes in cardiac electrical current conduction and, consequently, changes in the MEA direction. We aimed to define a longitudinal study to detect any changes in the direction of the MEA in Thoroughbred horses in training using ECG.

MATERIALS AND METHODS

Animals

A total of 43 Thoroughbred horses, 15 females and 28 males, were included in the present study. At the time of data collection, the horses were stabled at two racing stables in the San Rossore training centre (Pisa, Italy). All the horses were managed as follows: individually housed, fed twice daily with commercial forage and concentrates, and trained daily, except on Sundays.

Horses were selected for the following inclusion criteria: (1) remaining in the same stable, managed in the same way, throughout the study period; (2) aged between 2 and 4 years old; (3) no changes in the general objective clinical examination; (4) absence of abnormalities in resting ECG.

The horses included were then divided into three groups according to age (Table 1). This division was due to the different training programmes the horses underwent. The 2-year-old horses, not yet raced in competitions, were subjected to a gradual, less intense training programme than the 3- and 4-year-old horses. In addition, within the group of 2-year-old horses, 11% of the subjects (2/18) had not yet started any specific training programme at the time of the first electrocardiographic recording (T0).

ECG collection

ECGs were recorded with the horses at rest in their stalls. All subjects were approached by their caretaker and manually restrained by halter as per normal routine, without physical or pharmacological restraint. The experimenter then positioned the electrodes according to Dubois's method (da Costa et al., 2017) to measure the MEA in the frontal plane (Figure 1). The ECG trace was then recorded for a minimum of 2 min. The person who placed the electrodes was always the same (PP) for all the horses and in all the repetitions.

The electrocardiographic recordings were collected with a portable electrocardiograph (Ekuore six ECG leads, Chip Ideas Electronics SL, Spain), connected via an app (Ekuore Vet, Chip Ideas Electronics SL, Spain) to a smartphone (Galaxy A52s, Samsung, South Korea), digitised by the mobile device, archived in PDF format, and printed with a paper speed of 25 mm/s and an amplitude of 10 mm/mV. The traces of bipolar (DI, DII, DIII) and augmented unipolar (aVR, aVL, aVF) leads were then obtained.

The surface electrodes used were placed with alligator clips and electrically conductive gel (Farmacare®, Italy) to improve signal conduction.

TABLE 1 Number of horses by sex (female and male) and age (2, 3 and 4 years) and total number of horses included in the study ($N=43$).

Age (years)	Male	Female	Total No. for each age group
2	11	7	18
3	10	4	14
4	7	4	11
Total No. for each sex	28	15	43

ECGs were recorded three times for each subject in each age group, at day 0 (T0), 1 month (T1) and 2 months (T2) of training while the horses continued with their individual training programme.

Measuring the mean electrical axis

Intervals with easily identifiable, artefact-free QRS complexes in both DI and aVF were selected for each ECG tracing. The deflections of the QRS complex that developed above (positive deflection) and below (negative deflection) the isoelectric line between P and QRS were then measured with a ruler. The total deflections in each of the two leads were then calculated by subtracting the negative measurements from the positive ones. The value obtained was then reported on the hexaxial system. Perpendiculars were then drawn to the points marked on the respective vector reference lines, DI and aVF. The vector obtained, which joined the centre of the circle to the point of intersection of the perpendiculars, was identified as the mean electrical axis (MEA). The procedure was repeated for three QRS complexes for each horse within the same ECG trace, and the average of the values of the three MEAs obtained was then calculated.

Statistical analysis

The MEA values were tested for normality and were non-normally distributed (Shapiro–Wilk test: $W=0.80$; $p<0.001$). Median changes of MEA values were computed over the three evaluations (T0–T1, T0–T2 and T1–T2) for each age (2, 3, and 4 years) and sex (female, male).

The analysis of variance was performed using an unbalanced randomised block design model, using a Generalised Linear Mixed Model (GLMM; Glimmix procedure in the SAS/STAT 9.2; SAS Institute Inc.). In the model, the three ECG collection times (T0, T1

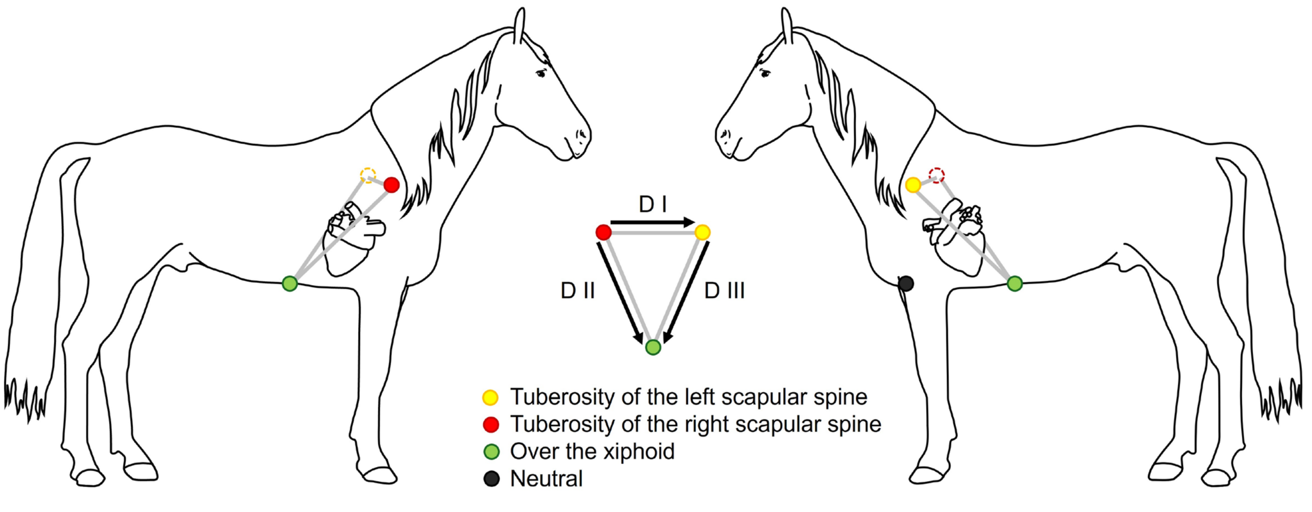

FIGURE 1 The electrodes positioned with the Dubois method (portions of the figure used images by Servier Medical Art, licensed under CC BY 4.0 DEED | Attribution 4.0 International | https://creativecommons.org/licenses/by/4.0/).

and T2), age (2, 3, 4 years) and sex (female, male), together with their interactions, were considered as fixed factors. The horse ID was considered a random factor, and MEA values were considered the dependent variable. Where GLMM revealed a significant effect, the Tukey–Kramer HSD (Honestly Significant Difference) test was used to investigate the possible differences between the MEA values in relation to the different fixed factors.

A multivariate approach was used to explore the data, thereby increasing their interpretability and enabling multidimensional data visualisation. We thus performed a PCA considering the different collection times for each horse as variables (Past 4.08).

RESULTS

Figure 2 shows the median changes in MEA values over the three evaluations for each age and sex. Changes in MEA seemed to occur particularly in 2-year-old horses, especially between T1–T0 and T2–T0 in females and between T2 and T0 in males (Figure 2).

The GLMM revealed a statistically significant effect of the variable time and the interaction between the two variables time and sex on the MEA. In contrast, no relationship was found between the MEA and sex, age and the other interactions between independent variables (time-age; age-sex; time-age-sex) (Table 2).

A statistically significant difference was found in the MEA values between T0 and T2 ($p < 0.01$; Table 3; Figure 3). Furthermore, the time-sex interaction appeared to impact the MEA values, with females showing a statistically significant difference in MEA values between T0 and T2 ($p < 0.05$; Table 4; Figure 3).

The PCA highlighted that the horse population, in terms of PC1 and PC2 values, was characterised by different patterns in MEA direction, with three horses (subjects A19, A24 and A26) having a higher variation, i.e. higher values of PC1 and/or PC2 (Figure 4). All relevant data are included (Item S1).

DISCUSSION

We measured the Mean Electrical Axis (MEA) three times, 1 month apart each time, in a population of Thoroughbred horses in training for flat racing. Our results suggest the variable "time" (2 months of training) influenced MEA orientation. This could be due to a change in cardiac morphology following training (i.e. hypertrophy), in line with investigations on exercise-induced cardiac hypertrophy detected

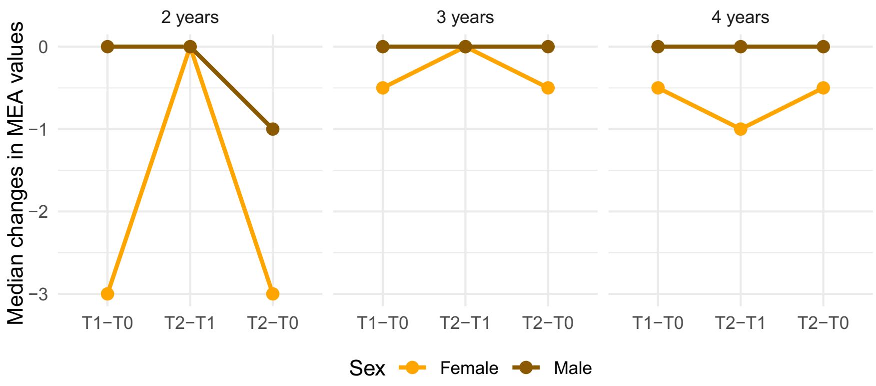

FIGURE 2 Line plot of the median individual changes in MEA values over the three evaluations (T0, T1 and T2) by age and sex.

TABLE 2 Effect of the independent variables (sex, age and time) and their interactions (time-sex, time-age, age-sex and time-age-sex) on MEA.

Variables	Numerator DF	Denominator DF	*F* value	Pr > *F*
Time	2	68.09	8.95	**<0.001*****
Sex	1	37	0.25	0.62
Time-Sex	2	68.09	3.46	**0.04***
Age	2	37	0.1	0.90
Time-Age	4	69.67	1.72	0.16
Age-Sex	2	37	1.66	0.20
Time-Age-Sex	4	69.67	0.65	0.63

Note: The independent variables with a statistically significant effect on MEA are in bold.
Abbreviation: DF, degrees of freedom.
*$p < 0.05$; ***$p < 0.001$.

by autopsy and echocardiography on horses (Cherdchutham et al., 2020; Kubo et al., 1974; Young, 1999; Young et al., 2005).

The variation in MEA orientation also appears to be influenced by factors other than training, with some horses showing individual variability in the MEA direction, as already reported in human medicine (Engblom et al., 2005; Schijvenaars et al., 2008). In humans, the MEA appears to vary according to certain individual characteristics, such as race, gender, age, weight, and chest configuration and to certain functional and physiological factors, such as pregnancy, posture or interindividual variability in how the Purkinje network of the heart is distributed (Engblom et al., 2005; Schijvenaars et al., 2008). The variation we found in the MEA orientation of some horses could therefore depend on interindividual variability and may not be detected in all horses in training.

TABLE 3 MEA values, expressed as the median, interquartile range (Q1–Q3), minimum and maximum, in relation to time (T0, T1 and T2).

Time	Median	Interquartile range (Q1–Q3)	Minimum/maximum
T0	**−79B**	**−82/−76**	**−86/−59**
T1	−80AB	−82/−76	−87/−59
T2	**−80A**	**−82/−77**	**−87/−59**

Note: In bold and with different letters are the MEA values resulting to be statistically different. Median values within the same column followed by different letters show significant differences (A, B: $p < 0.01$). Abbreviations: T, time.

The orientation values we obtained using Dubois's method confirm that the MEA in horses is directed to the left, cranially and dorsally (da Costa et al., 2017; Hesselkilde et al., 2021), unlike humans and small animals (van Loon & Patteson, 2010). These results are in agreement with the physiology of the cardiac conduction system in ungulates, in which the ventricular depolarisation wave proceeds from the apex to the base of the heart (van Loon & Patteson, 2010).

The results also indicate a statistically significant effect of sex-time interaction on the MEA, especially between T0 and T2 for females (Table 3). The influence of sex that we found is an exploratory finding and seems to disagree with what is reported in the literature, where males show a higher cardiac mass than females under the same training schedule (Young et al., 2005). daCosta et al. (2017) excluded the effect of sex on MEA, as did other studies on dogs (Gugjoo et al., 2014; Lerdweeraphon et al., 2020).

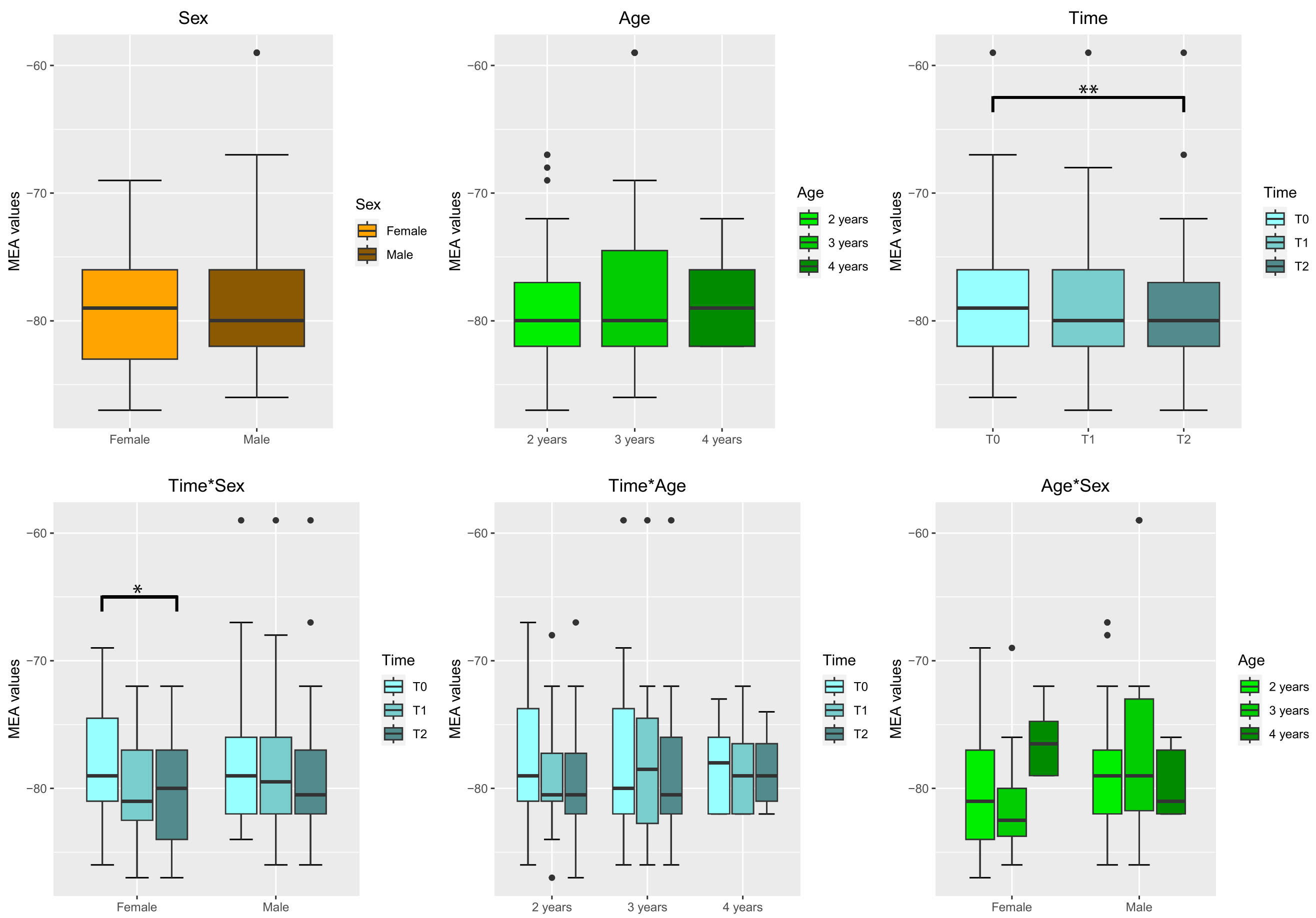

FIGURE 3 Boxplots of MEA values according to sex (female and male), age (2, 3 and 4 years), time (T0, T1 and T2) and their interactions (time-sex, time-age and age-sex). Statistically significant differences for time (between T0 and T2, $p < 0.01$) and time-sex interaction (for females between T0 and T2, $p < 0.05$) are indicated by two and one asterisks, respectively.

However, in our study too, the sex variable alone did not affect changes in MEA. It is therefore possible that the significance found for the sex-time interaction can be attributed predominantly to the time variable. In fact, sex correlates with time, as most of the females included in this study (7 out of 15) were part of the 2-year-old group, whose subjects had not yet started training and for whom a greater effect was expected. Alternatively, sex could have an influence on the MEA, which could be due to the earlier development of females compared to males (Trachsel et al., 2016). However, to date, this latter explanation remains a hypothesis and even in human medicine, the sex-dependent remodelling response of the heart in growing subjects is still poorly understood (Forså et al., 2023). Confirmatory studies will be needed in the future to support or refute our findings on the influence of sex on MEA in horses.

In our study, there also seems to be an individual component influencing the results obtained. The PCA identifies three outliers with values presenting the greatest variability of the whole group. This is in line with some studies conducted on humans (Engblom et al., 2005; Schijvenaars et al., 2008), where interindividual variability was found.

Our result is interesting because it suggests that individuality is one of the variables to consider in explaining the variations in ECG tracing as a function of cardiac adaptation to training. This could be useful in understanding that not all horses respond in the same way to the same type of training. Understanding how cardiac adaptation to training varies from individual to individual could be useful in optimising training and getting the best out of each horse athlete. Furthermore, this finding could suggest the influence of a genetic component in the cardiac adaptation to training (Watson, 2015). This could indicate that each horse can perform differently in different types of sport, according to its genetic characteristics, and that selection in this sense could be made to obtain certain desired characteristics and/or sports performance.

Our study has limitations due to the sex imbalance in the age groups of the study population (in 2-year-olds: 7 females and 11 males, in 3-year-olds: 4 females and 10 males and in 4-year-olds:

TABLE 4 MEA values, expressed as the median, interquartile range (Q1–Q3), minimum and maximum, in relation to time-sex interaction (T0-female, T1-female, T2-female; T0-male, T1-male, T2-male).

Time/sex	Median	Interquartile range (Q1–Q3)	Minimum/ maximum
T0F	**−79b**	**−81/−74.50**	**−86/−69**
T1F	−81ab	−82.5/−77	−87/−72
T2F	**−80a**	**−84/−77**	**−87/−72**
T0M	−79ab	−82/−76	−84/−59
T1M	−79.50ab	−82/−76	−86/−59
T2M	−80.50ab	−82/−77	−86/−59

Note: In bold and with different letters are the MEA values resulting to be statistically different. Median values within the same column followed by different letters show significant differences (a, b: $p < 0.05$). Abbreviations: F, female; M, male; T, time.

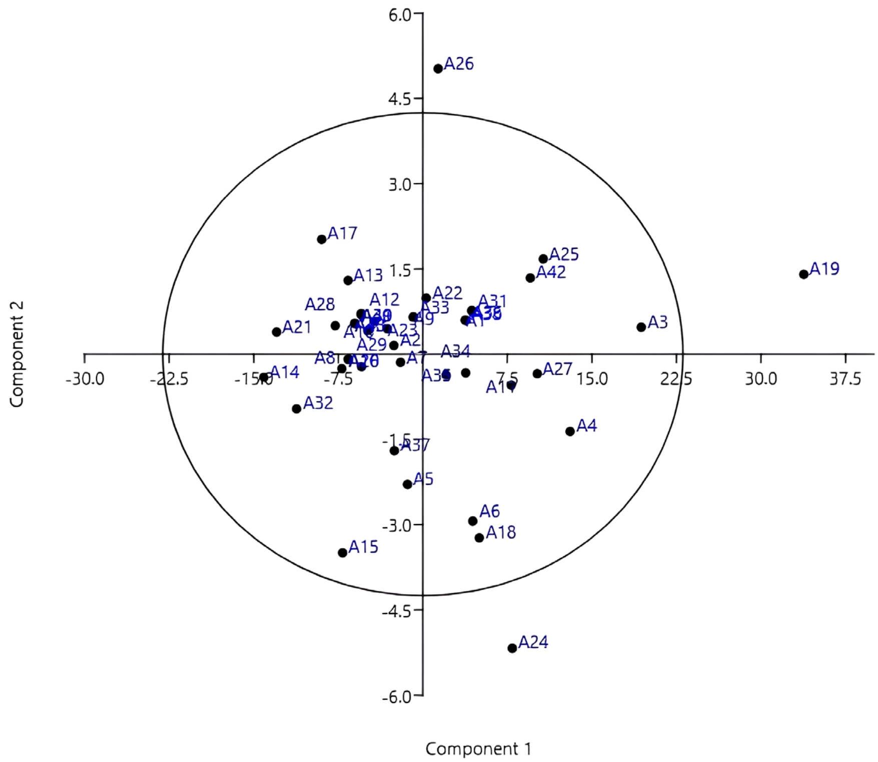

FIGURE 4 PCA scatter plot showing the individual distribution of subjects based on MEA values, in terms of the first Principal Components (PC1 and PC2). Subjects A19, A24 and A26 are outside the area that includes 90% of the subjects, showing a different pattern.

4 females and 7 males) and the number of horses within the categories (2-year-olds: 18/43 horses, 3-year-olds: 14/43 horses, 4-year-olds: 11/43 horses). These limitations are due to the inclusion criteria in the study and a protocol developed for use in the field, where it may be difficult to select balanced study groups according to individual characteristics (e.g. sex, age, etc.) without influencing normal management and training. Although in our work this limitation was handled with multivariate statistics, for future studies a more balanced sample would better define which variables affect the MEA.

In addition, we did not have control groups of subjects of the same age but not in training. The MEA variations that we found could be due to the normal growth of horses that have not yet completed their physical development. A timeline of 2 months seems a limited period to detect a cardiac variation due to physical growth alone. However, it would be advisable to extend the duration of future studies to follow the horses over a longer period, both to better assess the possible effects of training and to rule out the influence of growth.

Another limitation of our study could be the choice of a 1-month interval between ECGs. To the best of our knowledge, there are no data concerning longitudinal measurements of MEA on horses in training, and the appropriate interval for repeating measurements and verifying an actual training-induced change in MEA is unknown. Furthermore, our study lacks ultrasound confirmation of changes in cardiac morphology that may have influenced MEA values. The introduction of echocardiography, together with electrocardiography, may be beneficial in the study of MEA variations in different contexts.

Finally, for logistical reasons, we calculated the MEA directly on paper rather than from the raw data on the PC. However, if possible, it is recommended to calculate the MEA directly from the raw data and to use a higher amplitude to increase the accuracy. Despite these limitations, our study is the first to document a relationship between training time, sex, and individuality on MEA orientation. Confirmatory studies of our findings are required, particularly concerning the sex and individual differences we found in MEA values, but MEA may prove to be a simple, inexpensive, and readily detectable measure for investigating the cardiac response to exercise in horses and, consequently, for tailoring training regimes to the needs of the individual horse athlete.

CONCLUSIONS

To the best of our knowledge, these are the first results indicating that horse training triggers physiological changes in the heart detectable by ECG tracing and the analysis of the MEA. This is also the first exploratory study to suggest the influence of other variables such as sex and, above all, the individuality of the subjects. The action of these variables may have contributed to the difficulty in detecting training-dependent changes in the MEA to date.

CLINICAL RELEVANCE

- Mean electrical axis analysis may be useful in detecting exercise-induced changes in the horse's heart.
- Sex appeared to influence the changes in the mean electrical axis in response to training.
- Individuality seemed to play a key role in the cardiac adaptation of horses to exercise.

AUTHOR CONTRIBUTIONS

M. Felici: Data curation; methodology; writing – original draft; writing – review and editing. **P. Pratelli:** Data curation; methodology; writing – review and editing. **A. Gazzano:** Funding acquisition; methodology; writing – review and editing. **F. Cecchi:** Data curation; formal analysis; methodology; writing – review and editing. **G. Incastrone:** Methodology; writing – review and editing. **N. Bernabò:** Data curation; formal analysis; methodology; writing – original draft; writing – review and editing. **P. Baragli:** Conceptualization; methodology; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICS STATEMENT

The authors declare animal ethics approval was not needed for this study.

INFORMED CONSENT

Owners gave consent for their horses' inclusion in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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