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# Evaluation of clinical and electrocardiographic changes during the euthanasia of horses

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ABSTRACT

The objective of this prospective field study was to investigate whether commonly used criteria for clinical death occurred at the same time as cardiac death, as determined by electrocardiography. Specific ECG changes during euthanasia were also studied. Twenty-nine horses were euthanized with pentobarbital at two different dose rates and 15 of the 29 horses also received detomidine hydrochloride for sedation. ECG was recorded prior to and during euthanasia. Time to collapse, cessation of reflexes, heart sounds and asystole were recorded. ECG recordings were used to calculate RR intervals, PQ duration, QRS duration, distance from QRS complex to end of T wave corrected for HR ($QT_c$ interval), duration of T-wave from peak to end ($T_{peak}T_{end}$) and amplitudes of T wave ($T_{peak}$) before and during euthanasia. Differences between groups and ECG changes were evaluated using analysis of variance.

Clinical determination of death occurred before cardiac death ($P < 0.05$). Sedated horses took longer to collapse than unsedated horses ($P < 0.0001$), but asystole occurred faster in sedated horses ($P < 0.0001$). No significant changes in QRS duration were observed, but RR, PQ, $QT_c$, $T_{peak}T_{end}$ and $T_{peak}$ were influenced by both pentobarbital dose and sedation ($P < 0.05$–$<0.0001$). In conclusion, sedation prior to euthanasia resulted in a shorter time to asystole and is therefore recommended for the euthanasia of horses. Importantly, the results show that the clinical definition of death occurred significantly earlier than cardiac death (defined as asystole), which indicates that the clinical declaration of death in horses could be premature compared to that used in humans.



## Introduction

Euthanasia means 'good death' and is defined as death with minimal pain and distress (USA-AVMA-Panel, 2007). Euthanasia agents cause death by three basic mechanisms, namely, (1) hypoxia, (2) direct depression of neurons necessary for life functions, or (3) physical disruption of brain activity necessary for life. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and ultimately, the loss of brain function. Euthanasia of horses can be performed either by injection, or using a penetrating bolt, or by a free bullet (Jones, 1992).

In human medicine, two different definitions on death are used, cardiorespiratory death and brain death. These terms are especially important when considering organs for transplantation and when to perform resuscitation. However, there is continuous debate about these definitions and concerns are often raised about the accuracy of the clinical diagnosis of death (Kerridge et al., 2002; Doig and Burgess, 2003; Zamperetti et al., 2003). In veterinary medicine, the definition of death is not clear. The American Veterinarian Medical Association states that death should be confirmed by examining the animal for cessation of vital signs, but no exact definition of death is specified.[1]

Among the most important vital signs of death are the absence of a palpable or auscultable heartbeat, absence of the corneal reflex, and respiratory arrest (Sinclair, 2001). Although apparently simple, the evaluation of these vital signs is not an easy task and in human medicine, pulse palpation has now been omitted in modern cardiac arrest algorithms due to lack of sensitivity, even for experienced personnel. In ECG-monitored human patients, asystole is a marker for the onset of death. Although death is always associated with asystole, short term asystole can be reversible. In the setting of euthanasia, it is likely that prolonged asystole is associated with death.

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[1] See: www.avma.org/issues/animal_welfare/euthanasia.pdf (Accessed July 25, 2012).

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Several reports describe euthanasia of horses (Jones, 1992; Hubbell et al., 1993; Knottenbelt et al., 1994; Steere, 1990; Sinclair, 2001), but to our knowledge, no studies have investigated the electrical activity of the heart using systematic analysis of ECGs during euthanasia. Moreover, equine studies focusing on morphological changes in electrocardiographic wave patterns and subsequent clinical changes are limited (Ayala et al., 2000; Matthews and Hartsfield, 2004; Morgan et al., 2011) and most studies focus on the assessment of arrhythmias. Information on normal ECG morphology and altered morphology during the process of death are essential in building a database of ECG changes associated with various pathophysiological conditions in horses. Such information enables the equine clinician to recognize clinical patterns and intervene when significant ECG changes occur.

The purpose of the present study was to study ECG changes during the euthanasia of horses performed using sodium pentobarbital at two different dose rates, with or without sedation. Additionally, temporal associations between commonly used clinical criteria for death, such as clinical examination, auscultation, cessation of reflexes and collapse, and cardiac death as determined by ECG, were investigated.

## Materials and methods

### Animals

Twenty-nine horses (aged 1–30 years, bodyweight 160–703 kg) that were euthanized at University of Copenhagen were included in this project. The reasons for euthanasia included orthopaedic conditions, chronic incurable diseases and elective reasons. Horses euthanased due to cardiovascular problems, systemic illness, or exhaustion and horses euthanased during anaesthesia were not included. Prior to euthanasia, a clinical examination was carried out and informed consent for participation in the study was obtained from the horse owner. As the clinical procedures performed were standard procedures, permission was not required from the Danish Animal Experimentation Inspectorate.

### Euthanasia procedure

Horses were randomly assigned into four groups based on differences in pentobarbital dose (high or low dose), and whether the horses were sedated with detomidine hydrochloride (Domosedan, Orion Pharma) prior to euthanasia (Table 1). An intravenous (IV) catheter (12 G, Intraflon, Vygon, E-vet) was placed in the jugular vein to avoid perivascular injection of drugs. Euthanasia was induced by administration of pentobarbital at 66.67 mg/kg IV for the high dose, as recommended by Knottenbelt (1995). The low dose was 44.44 mg/kg administered IV as a 200 mg/mL solution. Sedation was administered IV 5–10 min before euthanasia at a dose rate of 0.01 mg/kg. An exception was made to random assignment to treatment groups for one nervous horse that needed immediate sedation. That horse was sedated using detomidine (0.02 mg/kg IM).

### Protocol

For all euthanasia procedures, one person registered the timing of events using a stopwatch and another person administered the pentobarbital and recorded the cessation of clinical signs. During the injection procedure, the horse was restrained by a third person, but a number of individuals performed this role throughout the study. The stop watch was started when the injection of pentobarbital was initiated and split times were registered on the stop watch for injection time, time taken for the horse to collapse (defined as when the horses were in lateral recumbency), time to absence of auscultable heartbeat, time to asystole (defined as a flat baseline on ECG) and time for loss of palpebral, corneal and perineal reflexes.

**Table 1**
Distribution of the horses into four groups.

| | Pentobarbital | Sedation with detomedine (0.01 mg/kg IV) |
|---|---|---|
| Group 1 ($n$ = 9) | High dose (66.67 mg/kg IV) | Present |
| Group 2 ($n$ = 6) | High dose (66.67 mg/kg IV) | Absent |
| Group 3 ($n$ = 6) | Low dose (44.44 mg/kg IV) | Present |
| Group 4 ($n$ = 8) | Low dose (44.44 mg/kg IV) | Absent |

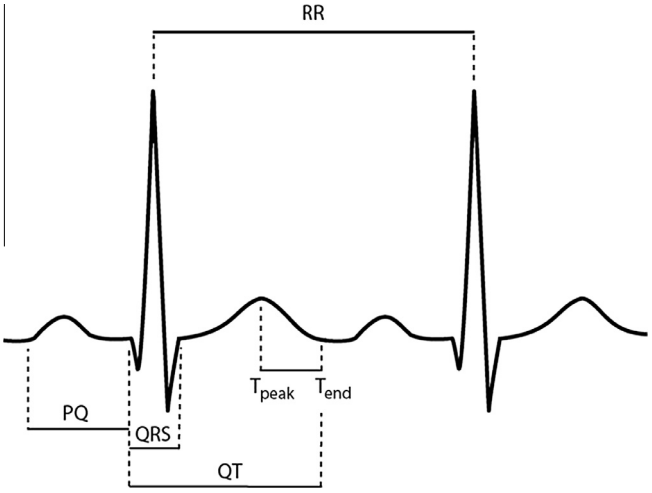


**Fig. 1.** ECG showing the different ECG variables. RR (ms), distance between two R waves; PQ (ms), distance from P to Q wave; QRS (ms), duration of QRS complex; QT (ms), distance from Q to the end of the T wave ($T_{end}$); $T_{peak}T_{end}$ (ms), duration from top of the T wave ($T_{peak}$) until the T wave reaches baseline ($T_{end}$); $T_{peak}$ (mV), amplitude of the T wave.

### Electrocardiographic monitoring

A modified chest lead ECG (Krutech Televet 100, Kruuse A/S) was recorded, monitored and stored telemetrically on a laptop. Hair coat was clipped to ensure good skin contact and electrodes were attached on the saddle girth area, the red and yellow electrodes on the left and right side respectively, approximately 20 cm from the dorsal thorax. The green electrode was placed 5 cm lateral to the sternum on the left and the black 0-electrode on the left side of the thorax, 15 cm below the red electrode.

ECGs were recorded prior to sedation and euthanasia and during euthanasia. ECG monitoring during euthanasia commenced at the time that pentobarbital injection was initiated. Monitoring ceased at 1200 s (20 min). As soon as asystole appeared and reflexes were absent, the animal was considered dead. In nine cases, perineal reflexes continued after asystole, but the animal was considered dead at the time of asystole.

Measurements of ECG variables were performed directly on the computer afterwards when all horses had been euthanized. RR intervals, PQ duration, QRS duration, distance from start of QRS complex to end of T wave (QT interval), duration of T-wave from peak to end ($T_{peak}T_{end}$) and T wave amplitude ($T_{peak}$; Fig. 1) were measured on lead II. These measurements were performed before euthanasia (time 0) and repeated every minute for the first 5 min after the commencement of pentobarbital injection (time 1, 2, 3, 4, 5 min), followed by measurements at 10 and 15 min after pentobarbital injection. If asystole occurred before 15 min, measurements stopped after asystole occurred. The QT interval was corrected for HR by Bazett's formula (Bazett, 1920):

$$QT_B = \frac{QT}{\sqrt{RR}}$$

and was named $QT_c$. All ECGs were manually checked for arrhythmias according to previously described definitions (Buhl et al., 2010).

### Statistics

Analysis of variance (ANOVA) using a general linear model was performed to evaluate the effect of pentobarbital dose (high/low) and sedation (present/absent) on time taken to collapse, auscultable heartbeat to cease, asystole to occur and reflexes to be lost. The effect of dose (high/low), sedation (present/absent) and time (time 0, 1, 2, 3, 4, 5, 10 and 15 min) on RR, PQ, QRS, $QT_c$, $T_{peak}T_{end}$ and $T_{peak}$ was also evaluated using ANOVA and a general linear model (PROC GLM, SAS 9.2, Statistical Analysis System). To compare differences between time 0 (before euthanasia) and after euthanasia (time 1, 2, 3, 4, 5, 10 and 15 min), Dunnett's test was used. As only one horse had electrocardiographic activity 15 min after pentobarbital injection, statistical analysis was not performed on data at 15 min after injection. $P$-values of <0.05 were considered statistically significant. Box-and-whisker plots were performed to illustrate the data (Graph Pad Prism version 5.04).

## Results

Mean injection time was 17 s (range 6–45 s). The allocation of horses to the four groups is shown in Table 1.