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ELECTROCARDIOGRAPHY OF GREVY'S ZEBRAS (*EQUUS GREVYI*)

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Abstract: Electrocardiograms (ECGs) are a good baseline test for assessing cardiac rhythm. ECGs have not been reported in any zebra species and in very few Perissodactyla species. Standard limb, six-lead ECGs were recorded in 23 anesthetized Grevy's zebras (*Equus grevyi*). Heart rate, RR interval, P-wave duration, RR maximum/minimum, PR interval, QRS duration, QT interval, ST segment deviation, P-wave amplitude, QRS amplitude, and T-wave amplitude were measured and calculated from lead II ECGs from these Grevy's zebras. Several variables were tested, including gender, age (0–24, 24–48, 48–180, and >180 mo), weight (<350 kg or >350 kg), pregnancy status, and anesthetic differences (standard dose or supplemented dose), to see if they affected ECG values in these animals. There were no significant differences in any of the ECG parameters between genders. RR and QT intervals were longer in older zebras; heart rates were faster in younger zebras. The RR and PR intervals, as well as the QRS duration, were greater in heavier zebras; heart rates were faster in lighter zebras. The RR interval was significantly longer in pregnant zebras. There were no significant differences in any of the ECG parameters for zebras anesthetized with a standardized dose of the drug combination etorphine–detomidine–acepromazine compared to those receiving additional supplements of these drugs and/or ketamine. All other parameters were not significantly different among groups, except where noted previously. The results of this research indicate that differences in ECG parameters in zebras may occur between animals of different ages, weights, and pregnancy status and that these factors should be considered when interpreting the respective ECGs of these zebras.
Key words: ECG, electrocardiogram, *Equus grevyi*, zebra, ungulate.

INTRODUCTION

An electrocardiogram (ECG) is a graphic recording of electrical potentials produced by the heart muscle during different phases of the cardiac cycle. Lead systems allow evaluation of the heart from different angles, with each different angle representing a different lead. Lead II is commonly used to assess rhythm. Though not utilized in this study, the base apex lead is commonly used in domestic equids because of its parallel alignment to the electrical axis, which allows greater deflection of the wave forms. ECG findings are generally employed to diagnose abnormalities in the cardiovascular system by assessing cardiac rhythm, chamber size, conduction abnormalities, and to aid in treatment monitoring.[3] For instance, a normal P wave originating from the SA node should be positive and rounded on lead II. An upright P wave that differs from the normal shape is indicative of an ectopic pacemaker in the atrium. P waves that are inverted on lead II are indicative of an impulse being formed in or near the atrioventricular junction. If the P wave is absent altogether, then atrial fibrillation or atrial standstill should be suspected. Buried P waves in QRS complexes are indicative of atrioventricular junctional rhythms. A P wave that is increased in amplitude or duration can indicate atrial enlargement.[3] These findings are consistent with heart disease in numerous vertebrate species, but the absence of reference material in Grevy's zebras precludes us from drawing similar conclusions. Although there are a number of studies evaluating cardiac morphology and function in domestic horses,[8] there have been no studies evaluating electrocardiography measurements in zebras (*Equus* spp.).

In one report, an ECG was performed on a 12-yr-old, male African black rhinoceros (*Diceros bicornis*), another member of the Perissodactylid family.[7] The heart rate of the animal was low (31 beats per minute [bpm]), as would be expected for an animal with a large body mass. Low voltages were recorded for all complexes, which were presumably related to the thickness of rhinoceros skin. Both the QT and PR intervals were prolonged.[7] Prolonged QT intervals have been reported in domestic equids but appear more commonly in other ungulates, including the giraffe, camel, and elephant.[2,4–6,8]

The purposes of the study were 1) to establish

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reference ECG parameters for the Grevy's zebra (*Equus grevyi*) and 2) to determine if gender, age, weight, pregnancy, or anesthetic differences have an affect on ECG values in these animals. Specific biological hypotheses tested in this study included the following: 1) there will be no difference in ECG parameters between genders; 2) ECG parameters will vary by age class, more specifically, between the youngest and oldest zebras; 3) ECG parameters will vary between animals based on weight when comparing zebras that weigh over 350 kg to zebras weighing less than 350 kg; 4) ECG parameters will be affected by pregnancy; and 5) ECG parameters will not differ for zebras given supplemental anesthetic agents after induction with a standardized drug combination.

MATERIALS AND METHODS

Electrocardiograms were performed on 31 captive Grevy's zebras (27 females and four males) that were anesthetized. The female zebras were housed together in a large outdoor enclosure (12 acres; 49,292 m^2), and male zebras were housed individually in smaller outdoor pens (3 acres; 12.95 m^2) at a private breeding facility for endangered species in northern Florida (USA). Concentrated feed sources and water were removed approximately 15 hr prior to immobilization, but animals were allowed to graze on their enclosure pasture ad libitum overnight. The zebras were moved into a small catch pen (1.3 acres; 5.26 m^2) for darting, and all subsequent procedures were conducted within that area. Initial drug dosing was based upon estimated weights. For the drug dose defined as ''standard,'' the combination of etorphine HCl (0.01 mg/kg; ZooPharm, Laramie, Wyoming 82070, USA), detomodine HCl (0.04 mg/kg; Dormosedan, Pfizer Animal Health, Exton, Pennsylvania 19341, USA), and acepromazine maleate (0.03 mg/kg; The Butler Company, Columbus, Ohio 43228, USA) was administered intramuscularly using a remote drug delivery system (Telinject Vario 3V Rifle with Telinject 3.0 ml Vario syringes and needles; Telinject USA Inc., Agua Dulce, California 91390, USA). Some zebras ($n = 2$) received ketamine HCl (0.4 mg/kg; Keta-Thesia, Burns Veterinary Supply, Inc., Westbury, New York 11590, USA) intravenously as part of a supplemental regimen to provide safe recumbency. After acquisition of animal weight, actual drug doses were calculated and showed some variation: etorphine ranged from 0.01 to 0.02 mg/kg, detomodine ranged from 0.02 to 0.06 mg/kg, and acepromazine ranged from 0.01 to 0.02 mg/kg.

All zebras in this study received total anesthetic doses between these ranges. A higher dose would occur when an animal received a higher mg/kg rate than intended because that individual weighed less than anticipated or if additional drugs (supplement) were needed to achieve an appropriate anesthetic plane. A lower dose occurred when an animal received a lower mg/kg rate than intended because the animal weighed more than expected. All initial drugs were given intramuscularly via dart; all supplemented drugs were given intravascularly. In cases in which only partial darts were administered, an estimation of the volume initially received and the additional volume given by a second dart were added, and the ranges above reflect the total dosage delivered.

Immediately after recumbency, the zebras were placed onto a large strip of plywood (1.2 m × 2.4 m) and weighed to the nearest kilogram. Supplemental oxygen (10 L/min via nasal insufflation) was provided during the procedure. Each animal received its annual diagnostic evaluation and preventive medicine procedures, including physical examination, blood collection from the jugular vein, vaccination, prophylactic antibiotic administration, dental float, and hoof trim. ECGs were recorded during these procedures; ECGs were recorded only when there was no apparent motion. All ECG readings were performed by the same person (DM) and were recorded with the zebra in right lateral recumbency, with forelegs and rear legs positioned perpendicular to the long axis of the body and parallel to each other.

ECG electrodes were attached to the skin, and isopropyl alcohol was applied at the electrode sites in a conventional fashion. ECGs were recorded with a six-lead (lead I, II, III, avR, avL, and avF) handheld device (Vet Biolog; Micromedical Industries Ltd., Labrador, Queensland, Australia) in which heart rate, RR interval, P-wave duration, maximum–minimum RR, PR interval, QRS duration, QT interval, ST segment deviation, P-wave amplitude, QRS amplitude, and T-wave amplitude were measured and calculated. ECGs were standardized at 1 mV = 10 mm with a paper speed of 25 mm/sec. The heart rate, duration, and amplitude values were determined utilizing averages from lead II. The number of values that were averaged varied from zebra to zebra depending on the length of time that the ECG was recorded; approximately 12 waveforms was average. The ECG device computed averages of all the waveforms obtained to calculate the results. Calculations were made from data that were obtained only from lead II.

After completion of procedures, antagonists were administered to the animals based on the exact weight of each obtained during the procedure: naltrexone HCl (0.6 mg/kg; ZooPharm), yohimbine HCl (0.05 mg/kg; ZooPharm), and tolazoline HCl (0.27 mg/kg; Tolazine, Lloyd Laboratories, Shenandoah, Iowa 51601, USA). All antagonists were given half intravascularly and half intramuscularly. Recoveries were uneventful.

Statistical analysis

Descriptive measures, including the mean, median, standard deviation (SD), 10% and 90% quartiles, and minimum (min) and maximum (max), were calculated for all ECG parameters, as well as for the weight and age. The distribution of the data was evaluated using the Shapiro–Wilk test. The mean, SD, and min and max values were reported for normally distributed data, while the median, 10% and 90% quartiles, and min and max values were reported for non–normally distributed data. Five different independent variables were evaluated for this sample population: gender, age, pregnancy status, weight, and total anesthetic dose of the drug combination administered. The zebras were divided into four distinct age groups: 0–24 mo (class 0: $n = 3$), 24–48 mo (class 1: $n = 2$), 48–180 mo (class 2: $n = 11$), and greater than 180 mo (class 3: $n = 7$). This age categorization was based on previous work that classified age in Perissodactyla.[1,10,13,17] Pregnancy was determined via rectal palpation; those females determined to be pregnant were classified into a separate category. Zebras were also divided into two different weight classes: <350 kg ($n = 10$) and >350 kg ($n = 13$). Adult Grevy's zebras generally weigh between 350 kg and 450 kg. Zebras were also categorized into two groups based on drug dosing: standard dose ($n = 20$) or supplemented dose ($n = 3$).

For normally distributed data, differences in ECG parameters were evaluated by gender, age, pregnancy status, weight, and total anesthetic dose using a one-way analysis of variance (ANOVA). For the age category in which the independent variable had greater than two categories, a Tukey's test was used to further assess for differences among groups. Data that did not follow a normal distribution were evaluated using the Mann–Whitney test (MW) or the Kruskal–Wallis test (KW) (>2 categories). When evaluating gender, weight, and pregnancy status in females, analysis was also performed while controlling for age using a two-way ANOVA. A general linear model was used to determine if there were any biological interactions. SPSS 11.0 (SPSS, Inc., Chicago, Illinois 60629, USA) was used to analyze the data. A P value of ≤ 0.05 was used to determine statistical significance. A power analysis was performed for those data in which a significant difference was not found.

RESULTS

ECGs were performed on 31 Grevy's zebras. The ECG recordings of eight (26%) of the zebras could not be used as a result of ECG abnormalities and motion artifact noticed postimmobilization. The remaining 23 zebras were used to establish reference parameters for apparently healthy Grevy's zebras. The majority of the zebras were females (91%, 21/23), with fewer males (9%, 2/23). The average age of the zebras was 129 mo (SD, ±94.1; min = 5 mo; max = 337 mo). The median weight for the zebras was 359.5 kg (10–90% quartiles = 134–404; min = 9 kg; max = 473 kg). Two (9.5%, 2/21) of the females were pregnant.

There was no significant difference in any of the ECG parameters between genders: heart rate (HR; bpm, $F = 0.03$, $P = 0.8$); RR interval (ms, $F = 0.4$, $P = 0.5$); P-wave duration (ms, $F = 1.4$, $P = 0.2$); max–min RR (ms, $F = 3.9$, $P = 0.06$); PR interval (ms, MW: 9.5, $P = 0.2$); QRS duration (ms, $F = 0.01$, $P = 0.9$); QT interval (ms, $F = 0.8$, $P = 0.4$); ST segment deviation (μV, $F = 1.6$, $P = 0.2$); P-wave amplitude (mV, $F = 0.155$, $P = 0.7$); QRS amplitude (mV, $F = 0.2$, $P = 0.6$); and T-wave amplitude (mV, $F = 0.1$, $P = 0.8$).

There was a significant difference in the HR ($F = 4.8$, $P = 0.01$), RR interval ($F = 6.2$, $P = 0.02$), and QT interval ($F = 7.1$, $P = 0.01$) between age classes. Heart rates were significantly slower in the two oldest classes of zebras (age class 2: $P = 0.02$; median = 62 bpm; 10–90% quartiles = 46.6–96.2; min = 45 bpm; max = 101 bpm; age class 3: $P = 0.007$; median = 60 bpm; 10–90% quartiles = 42.0–74.0; min = 42 bpm; max = 74 bpm) compared to the youngest class (age class 0) of zebras (median = 111 bpm; 10–90% quartiles = 78.0–110.0; min = 78 bpm; max = 120 bpm). The RR interval was significantly longer ($P = 0.03$) in the oldest zebras (class 3, greater than 180 mo: mean = 1,025.1 ± 213.3 [SD] ms, min = 807 ms; max = 1,442 ms) compared to the youngest zebras (class 0, 0–24 mo: mean = 603.0 ± 144.2 [SD] ms; min = 501 ms; max = 768 ms). The QT interval was significantly longer in the two oldest classes of zebras (age class 2, 48–180 mo: $P = 0.03$; mean = 518.0 ± 66.3 [SD] ms; min = 427 ms; max = 613 ms; age class 3, >180 mo: $P = 0.02$; mean = 543.3 ± 104.2 [SD] ms; min = 443 ms; max = 773 ms) compared to the youngest class of zebras (mean = 346.7 ± 127.1 [SD] ms; min = 223 ms; max = 477 ms). There was no significant difference in the P-wave duration ($F = 3.8$, $P =$

Table 1. Electrocardiogram parameters by weight class in anesthetized Grevy's zebras (n = 23). Parameters in the table were found to have statistically significant differences between animals weighing greater than or less than 350 kg.

Parameter	Weight (kg)	Mean	Standard deviation	Minimum	Maximum
Heart rate (beats/min)	<350	81.2[a]	24.4[b]	53.0	120.0
	>350	63.1[a]	9.0[b]	42.0	74.0
RR interval (ms)	<350	807.8	220.3	501.0	1,124.0
	>350	970.0	179.0	807.0	1,442.0
PR interval (ms)	<350	170.4	38.9	107.0	210.0
	>350	194.5	16.9	153.0	210.0
QRS duration (ms)	<350	86.5	30.6	50.0	137.0
	>350	109.0	21.2	80.0	143.0
QT interval (ms)	<350	476.9	126.8	223.0	660.0
	>350	512.5	82.2	437.0	703.0

[a] Median.
[b] 10–90% Quartiles.

0.07), max–min RR (KW: 5.0, P = 0.3), PR interval (KW: 4.0, P = 0.2), QRS duration (F = 0.6, P = 0.5), ST segment deviation (F = 0.2, P = 0.6), QRS amplitude (F = 1.2, P = 0.3), or T-wave amplitude (F = 0.08, P = 0.8) between age classes.

The HR (F = 5.2, P = 0.03), RR interval (F = 4.2, P = 0.05), PR interval (F = 4.6, P = 0.04), and QRS duration (F = 4.4, P = 0.04) were significantly different between the two weight classes (Table 1). There were no significant differences in the P-wave duration (F = 1.6, P = 0.2), max–min RR (F = 0.9, P = 0.3), QT interval (F = 1.2, P = 0.2), ST segment deviation (F = 0.7, P = 0.3), P-wave amplitude (F = 0.1, P = 0.7), QRS complex amplitude (F = 3.6, P = 0.07), or T-wave amplitude (F = 0.4, P = 0.835) between weight classes.

The RR interval was significantly longer in the two pregnant female zebras (F = 5.7, P = 0.03; mean = 1,188.5 ± 205.6 [SD] ms; min = 1,043 ms; max = 1,334 ms) compared to 19 nonpregnant females (mean = 882.5 ± 170.1 [SD] ms; min = 501 ms; max = 1,124 ms). All other parameters between pregnant and nonpregnant zebras were unremarkable.

There were no significant differences in any of the ECG parameters between the zebras that received the standard dose and those that received supplemental anesthetic doses, including the HR (F = 1.1, P = 0.3), RR interval (F = 0.02, P = 0.9), P-wave duration (F = 0.4, P = 0.5), max–min RR (MW = 13.5, P = 0.8), PR interval (MW = 14.5, P = 0.9), QRS duration (F = 0.4, P = 0.5), QT interval (F = 0.01, P = 0.9), ST segment deviation (F = 0.001, P = 0.9), P-wave amplitude (F = 0.06, P = 0.09), QRS amplitude (F = 3.6, P = 0.08), and T-wave amplitude (F = 3.0, P = 0.1).

There were no significant differences in the P-wave duration, max–min RR, ST segment deviation, P-wave amplitude, QRS amplitude, or T-wave amplitude noted for any of the comparisons; therefore, the results of each of these ECG parameters were combined and are listed in Table 2. There were no significant biological interactions. Power analysis was performed in those cases in which no

Table 2. Reference ranges for anesthetized Grevy's zebra (n = 23) electrocardiogram (ECG) parameters. Parameters presented did not show any statistical differences for any of the variables studied (gender, age, weight, pregnancy status, and total anesthetic dose delivered).

Parameter	Mean	Standard deviation	Minimum (min)	Maximum (max)
P-wave duration (ms)	85.0	17.1	40.0	120.0
Max-min RR (ms)	23.0[a]	17.9–61.2[b]	17.0	54.7
ST segment deviation (μV)	14.2	61.7	−118.0	98.0
P-wave amplitude (mv)	0.22[a]	0.14–0.38[b]	0.1	1.0
QRS amplitude (mv)	0.7	0.3	0.3	1.1
T-wave amplitude (mv)	0.7	0.4	0.1	1.5

[a] Median.
[b] 10–90% Quartiles.

significant differences were found, and in all of these cases the power was <0.44.

DISCUSSION

Zebras are most closely related to other members of their suborder, Hippomorpha, which includes donkeys, horses, and ponies. Rhinoceros and tapirs belong to the suborder Ceratomorpha within the same order, Perissodactylidae.[16] Therefore, even though rhinoceros and tapirs are also Perissodactylids, they are not as closely related to the zebra as the zebra is to the domestic horse. Although ECGs in the donkey, pony, and domestic horse and, to a lesser extent, the rhinoceros have been evaluated, no studies have examined ECGs in zebras or tapirs.[7,10,11] Rhinoceros have lower heart rates, long P-wave durations, long PR and QT intervals, and greater T-wave amplitudes compared to the Hippomorpha species.[7] The zebra also has a long P-wave duration and a long QT interval. The QT interval is the summation of ventricular depolarization and repolarization, representing ventricular systole. Most of the ungulate species studied (e.g., giraffe, camel) have long QT intervals and, hence, a prolonged time for cardiac contraction and repolarization.[4,5,12] The P wave represents atrial depolarization and its duration indicates the time required for an impulse to pass from the sinoatrial (SA) node to the atrioventricular (AV) node. Therefore, the larger the atria, the longer the time required for the electrical impulse to traverse from the SA to the AV node. Canine species, for instance, have P-wave durations of 40 ms, compared to the zebra at 89 ms and the rhino at 100 ms.[4,7] The giraffe and the zebra have the shortest PR interval of the ungulate species examined. The PR interval reflects the activation of the AV junction. The longest PR intervals are recorded in the largest species, indicating that larger animals have AV nodes that are larger and, hence, that take longer to traverse or indicating that an increased length of time is needed to allow adequate emptying of the atria, which may be longer in larger animals.

Age, breed, and gender are important factors in determining HR and ECG parameters in horses; zebras appear to follow suit.[3] When examining ECGs for equine species, groups are divided into four different age classes based on physiologic data: <2 yr of age, 2–4 yr of age, 4–15 yr of age, and >15 yr of age.[1,10,13,17] As the average life span of a Grevy's zebra is 35 yr, or 420 mo, their longevity is comparable to that of other zebra and equine species.[9,12,16] Morphologic changes in the heart associated with growth, such as cardiac muscle mass and rotation of the electric cardiac axis, can continue until the animal is up to 2 yr in age. As equids age, HR decreases, and there is an increased influence of vagal tone.[1] Also, in equids there tends to be an increase in certain electrical patterns (e.g., increased amplitudes around 3–4 yr of age).[1] In adult animals, those over 4 yr of age, electrical configurations become more consistent.[1] In Grevy's zebras, there was a longer RR interval in the oldest class (class 3) when compared to the youngest class (class 0). This current study indicates that as the body size increases, the length of a single cardiac contraction increases, which in turn reduces the overall number of heartbeats per minute. Indeed the heart rates were slower for classes 2 and 3 compared to class 0. The QT interval was also longer in classes 2 and 3 compared to class 0. Older zebras seem to be more prone to heart disease. In this study, ECGs were performed on 31 Grevy's zebras, three (10%) of which were found to have heart disease. These animals were in the two oldest class divisions, a finding that is similar to those described in horses.[13] One animal in the study had an ectopic beat and the other two animals had systolic heart murmurs. Ectopic beats can be insignificant in horses and perhaps in zebras. The goal of this study, however, was to establish reference ranges for clinically healthy animals, so a full ECG was not recorded on these zebras and, hence, they were removed from the study.[9]

Two (9.5%; 2/21) of the females were pregnant and in their third trimester. The only significant difference between pregnant and nonpregnant female zebras was the RR interval. Since pregnant animals carry the additional weight associated with the fetus, the results showing a lengthened RR interval were not unexpected. However, for the same reason, it was expected that other parameters would also differ based on pregnancy status. With only two pregnant females in this present study, further work may be needed to adequately address the effect of pregnancy on the ECG. Also, as it can be difficult to assess early pregnancy by palpation, some animals may have carried first-trimester pregnancies without detection. Similarly, there were no differences in ECG parameters between genders. One might expect male zebra to be larger in size and, thus, might expect ECG parameters to vary by gender. Unfortunately, there were only two adult male zebras (90 kg and 370 kg) evaluated in this study. A review of the power analysis indicates that a larger sample size would be required to detect a difference, and one should not assume, based on these results, that pregnancy or gender does not affect these other parameters.

The weight divisions in Grevy's zebras also reflected similar findings. All the Grevy's zebras in the study were classified as being in good body condition, and none of the animals were classified as obese or thin. Adult Grevy's zebras generally weigh between 350 kg and 450 kg; zebras were thus divided into two different weight classes: <350 kg and >350 kg. Evaluation of weight classification was performed because as animals mature and their conformation changes to a higher percentage of muscle mass, the heart might be expected to work harder, which could affect some of the ECG values.[17] For the Grevy's zebras, the RR interval, PR interval, and QRS duration were all longer in the heavier (>350-kg) animals. Again, the PR interval is tied to atrial size, and larger atria would be expected in a larger-bodied animal. The RR interval is also lengthened as body mass increases, reflecting the increased time for a cardiac contraction. The QRS duration, which represents ventricular depolarization, is also lengthened as body mass increases. Heart rates were lower in the >350-kg animals than in the <350-kg animals, and this finding is consistent with those in other ungulate species.

When using a remote delivery system, animals sometimes receive only partial volumes/dosages of prepared darts, and they occasionally require supplemental anesthesia for safe handling. Furthermore, with free-ranging animals, one cannot measure the weight of the animal prior to calculating the dose of anesthetic. Hence, doses are calculated based on experience and previous medical records. Anesthetics can have a profound effect on the heart, and, therefore, supplemented doses or overdoses would similarly be expected to potentially affect cardiopulmonary variables such as blood pressure, arrhythmias, respiration rates, and central nervous system effects. Likewise, alterations in ECG readings may result from excitement in animals requiring multiple darts for immobilization. Although not evaluated in wild equids, changes in the ECG have been recorded in domestic horses between readings taken at rest and during exercise.[14] In horses, anesthetics themselves can alter ECG recordings, changing the HR and QT intervals.[15] Therefore, zebras receiving a second dose or a higher dosage than that provided to other zebras could potentially have altered ECG parameters. To explore this variable, the mg/kg rate of each dose was measured to the zebra's actual weight, and no significant differences in animals that received higher or lower doses or that received supplemental ketamine were found when their values were compared to those of zebras receiving the standard combination dose.

In conclusion, age, pregnancy status, and weight have an effect on ECG parameters in Grevy's zebras. These data represent the first ECG reference ranges for a zebra species and may be used by veterinarians to monitor zebras under anesthesia for procedures and to determine if zebras have ECG abnormalities.

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