Standardisation of electrocardiographic examination in corn snakes (*Pantherophis guttatus*)

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**Abstract**

**Introduction** Corn snakes are a very common pet reptile species, yet there is an absence of evidence-based literature standardising collection of ECG or detailing ECG deflection morphology in the normal animal. The authors describe a well-tolerated, reproducible technique and detail the cardiac cycle in terms of lead 2 equivalent waveforms and intervals.

**Animals** 29 adult corn snakes.

**Materials and methods** This prospective study evaluated, under species-appropriate, standardised conditions, a technique for producing standard six-lead ECG tracings. Lead 2 equivalent cardiac cycles were described in detail and statistically analysed for sex, weight, length, heart rate and mean electrical axis.

**Results** High-quality tracings demonstrated common ECG characteristics for this species, including no Q, S or SV waves, prolonged PR and RT intervals, rhythmic oscillation of the baseline, short TP segments, and a right displaced mean electrical axis. An influence of sex, weight or length on heart rate and mean electrical axis was not identified.

**Conclusions** To the authors’ knowledge, this is the first study to describe a standardised technique for recording ECG in significant numbers of normal corn snakes. Ranges have been provided that may be of diagnostic value or form the basis for future development of reference intervals for this species.

**Introduction**

Evaluation of cardiac performance and diagnosis of cardiac disease in the ophidian patient have historically received little attention, even in captive reptile species, such as corn snakes, which are common both in private and zoological collections.

For captive reptiles poor husbandry is ubiquitous and pervasive, and could predispose to cardiac disease either through immunocompromised or nutritional/metabolic derangement.1,2 Indeed, both primary and secondary forms of cardiac disease are widely described in the literature and demonstrate the broad spectrum of conditions from which snakes may suffer.3–12 Despite this, the prevalence of cardiac disease in snakes is unknown, and understanding of the impact of cardiac disease on morbidity and mortality in even very common pet species, such as corn snakes, is rudimentary in comparison with more familiar companion mammals.

Identification of cardiac disease by both the owner and the clinician can be very challenging. The typically sedentary lifestyles and highly restricted captive environments of many pet snakes place little demand on the cardiovascular system. A low basal metabolic rate and high tolerance for anaerobic respiration mean that heart disease is usually very far advanced before it becomes clinically apparent.3,13 Basic cardiac assessment, such as auscultation, has limited value due to poor contact between the stethoscope and the scales, while more advanced investigations, such as radiography, ECG and echocardiography, suffer from a paucity of species-specific reference ranges and standardised methodologies.13–14
In common with other non-crocodilian reptiles, the ophidian heart is a three-chambered structure composed of two atria and a single, common ventricle. A reptile-specific, prefilling chamber, the sinus venosus, is situated caudal to the right atrium and contains spontaneously depolarising pacemaker cells under vagosympathetic control. There is no sinoatrial node, atrial-ventricular node or His/Purkinje network that forms the specialised electrical conduction system of higher vertebrates, and no annulus fibrosus exists to provide an insulating plane between the atria and the ventricle. Instead a canal or ‘funnel’ of atrioventricular myocardium, which resembles the ill-defined atrioventricular (AV) node region of embryonic mammals and birds, serves as a conduit for electrical conduction into the ventricle. Despite these structural differences, the reptilian ECG waveform is morphologically similar to the mammalian, with P wave, QRS complex and T wave representing sequential atrial depolarisation, ventricular depolarisation and ventricular repolarisation, respectively. ECG is widely used in traditional companion mammal species to aid in the evaluation of cardiac health and to monitor cardiac rhythm while under general anaesthesia, and should also represent an attractive option for common captive snakes to both specialists and general practitioners. Unfortunately, very little literature exists detailing a standardised approach to recording ECG in snakes or describing wave amplitudes or segment durations. Previous research has typically involved a variety of different study designs, very small study cohorts or mixed species collections, and data sets cannot be extrapolated from companion mammals or even other reptilian species. The authors sought to correct this deficiency in a common pet species, the corn snake, and provide information useful to cardiac assessment.

The authors hypothesised that ECG can be easily recorded from conscious corn snakes in a non-invasive and well-tolerated way, and that they take on a recognisable sequence of PQRS deflections amenable to measurement. To investigate this hypothesis, a standardised technique for recording and measuring ECG was employed.

**Materials and methods**

The study population comprised 29 adult corn snakes (*Pantherophis guttatus*) (19 males and 10 females) from privately owned sources or a locally based charity rehoming centre, fed whole prey items on a seven-day cycle. As specific ages were unknown, snakes were classified as adult based on size (bodyweight and snout-to-vent length). Sex was determined by use of a metal probe passed into the hemipenis or musk sac (female if probe depth <5 scutes, male if >6 scutes). Owners were asked to withhold food for at least four days before hospitalisation. None of the snakes was undergoing ecdysis at the time of ECG recording.

Subjects were deemed healthy based on clinical history and physical examination which comprised evaluation of demeanour, body condition, respiratory rate and effort, coelomic palpation, and integumentary, oral and cloacal assessments. To allow for thermal acclimation and minimise the influence of travel stress, subjects were housed individually in reptile vivaria for at least four hours before ECG examination. Ambient vivarium temperature gradients ranged from 26°C to 30°C, which was considered to be within the preferred optimum temperature zone for this species. The ECGs were performed at a room temperature of 22°C–24°C as soon as the snakes were removed from the vivaria. Subjects were supported in ventral recumbency by a single assistant without the use of chemical restraint. When physical restraint was required, it was limited to gentle manual restriction of movement until the subject settled (figure 1). The heart was estimated at around 25 per cent snout-to-vent length, then either identified visually, with ultrasound confirmation, or found directly with the probe and coupling gel (figure 1). Non-traumatic adhesive ECG pads (Ambu BlueSensor N) were applied to the lateral skin surface in a four-lead system, 1 cm cranial and 1 cm caudal to the heart (figures 1 and 2). Attachment of the ECG pads to the subject’s scales was simple and reliable, and removal postrecording was achieved either with gentle manual traction or the use of a proprietary spray designed for adhesive bandage removal (Eaze-Off, Millpledge Veterinary). In both instances, removal was quick and atraumatic.
Due to the absence of limbs, positioning of the electrodes was based around a modified Einthoven’s triangle. The red electrode was placed on the right side and cranial to the heart, the yellow on the left and cranial to the heart, and the green on the left and caudal to the heart (figure 2). The ECG was recorded continuously for three minutes using both 25 and 50 mm/s at sensitivities of 10 and 20 mV.

Standard ECG measurements were made from the lead 2 equivalent (negative electrode right and cranial to the heart, positive electrode left and caudal to the heart), with the mean and sd calculated from a representative run of three consecutive cardiac cycles, using paper speeds of 50 mm/s and sensitivities of 20 mV. Heart rate (HR) was calculated from the number of R waves in a randomly selected 30-second period. The mean electrical axis (MEA) was extrapolated from the sum of the amplitudes of positive and negative deflections in leads 1 and 3 (lead 1 is defined with the negative electrode placed on the right and cranial to the heart and the positive electrode on the left and cranial to the heart; lead 3 is defined with the negative electrode placed on the left and cranial to the heart and the positive electrode on the left and caudal to the heart).

Study-specific measurement points were established to facilitate interval and amplitude calculations. An isoelectric baseline was selected at the segment immediately following the T wave to address the persistent deviation from horizontal at the PQ and ST segments (figure 3). As no clear Q or S waves were identifiable on any of the tracings, the QRS was referred to as an R wave, and the QT interval was measured from the start of the R wave to the end of the T wave and referred to as the RT interval (figure 3).

Data are expressed as mean±sd; statistical analysis was performed using commercial software (Minitab V.17, Coventry, UK). Data were tested for normality (Anderson-Darling test), and correlations between length or bodyweight and HR or MEA were determined by Pearson’s (normal) or Spearman’s rho (not normal) tests. Differences between sex and bodyweight and length were determined by a two-sample t test. For all tests, P<0.05 was considered significant.

Results

The process of cardiac identification using ultrasound probe and coupling gel was simple, accurate and rapid. Subject acceptance of handling, scanning and placement of self-adhesive ECG pads was typically excellent, with the exception of a subset of individuals that required additional time to settle before commencing the ECG. High-quality ECG tracings were obtained in all subjects.

A sinus rhythm was recorded in all of the snakes, with R wave preceded by a P wave and followed by a T wave. P wave morphology varied slightly between leads, but was typically low in amplitude, short in duration and monophasic. On lead 2, 15 of 29 (52 per cent) study subjects had negative P waves, while in 2 of 29 (7 per cent) biphasic P waveforms were present (figure 4). Q and S waves were not identified on any lead on any of the tracings; therefore, ventricular depolarisation consisted universally of monophasic R waves, with a high amplitude, short duration and positive deflection on lead 2 (figure 4). T waves were highly variable between individuals, with 20 of 29 (69 per cent) snakes exhibiting a negative waveform on lead 2. Otherwise, T waves were monophasic and commonly of moderate to low amplitude (figure 3).

There was a gradual downward sloping of the PR segment in 24 of 29 (83 per cent) snakes, while the RT segment followed an upward slope in 20 of 29 (69 per cent) snakes, making it difficult to establish the starting point of the T wave. PR and RT intervals were proportionally long, while TP segments were short. Of the 29 snakes in this study, 11 (38 per cent) demonstrated either pronounced shortening of the TP segment or early merging of the T and P waves (figure 5).
Lead 2 ECG amplitude and interval duration values are shown in table 1.

Statistics
There was no difference in bodyweight or length measurements between males and females (P=0.634 and P=0.506, respectively; figure 6A,B). HR was always between 20 and 97 beats per minute and was not found to be significantly different between sexes (P=0.094; figure 6D) or correlated with weight or length (P=0.972 and P=0.396, respectively; figure 7). MEA was always positive and displaced to the left, and was neither correlated with bodyweight nor length (P=0.910 and P=0.771, respectively; figure 8) or influenced by sex (P=0.233, T-value=1.24, degrees of freedom (DF)=15; figure 6C).

The mean and sd for weight, length, HR and MEA, by group, are presented in table 2.

Discussion
To the authors’ knowledge, this is the first comprehensive descriptive study of ECGs from fully conscious and unrestrained corn snakes. The authors used a modified Einthoven’s triangle that was atraumatic and well tolerated to generate high-quality recordings that were suitable for interpretation.

Despite the absence of a specialised electrical conduction network, ECGs from all corn snakes in this study consisted of sequential atrial and ventricular...
depolarisations analogous to the P wave, PQ interval, QRS complex and T waves observed in mammals. In contrast to mammals, however, and as has been previously reported in reptiles, none of the ECGs had recognisable Q or S waves at standard sensitivities, suggesting that PR and RT intervals rather than PQ and QT intervals are more appropriate terminology in this species.

The authors identified several key features of the PRT sequence that are relevant to future clinical and research use in this and potentially other snake species. First, P waves have heterogeneous polarity between individuals and can be positive, negative or biphasic (figure 3). It is also noteworthy that P waves were not preceded by sinus venosus waves, which have been variably identified in other reptile studies. Cardiac mass in reptiles is disproportionately small when compared with mammals or birds, representing only 0.2–0.3 per cent of body mass. The authors speculate that action potentials (AP) from this myocardial structure may be masked by preceding T waves and/or background APs from skeletal muscle contraction.

Secondly, although an AV node has not been identified in snakes, the PR interval, and hence atrioventricular conduction, is prolonged in a similar way to that mediated by the AV node in mammals. It has been suggested that a long PR interval in reptiles could reflect slow AP conduction along tortious pathways through the AV canal, slow rates of cardiac myocyte depolarisation or an absence of fast-propagating gap junctions from the AV canal. None of these mechanisms has been investigated in the corn snake but do represent possible areas for future research. The PQ interval in mammals is under autonomic control, and small changes in such a long PR interval in snakes could represent an easily measurable marker of autonomic status and metabolic health.

Thirdly, R waves were the tallest complexes of the sequence and always positive on lead 2, findings which suggest homology to mammals by demonstrating a rapid systolic phase and AP propagation towards lead 2, respectively. This feature also provides an easily identifiable waveform when interpreting corn snake ECG tracings (figure 3).

Fourthly and finally, the T wave was commonly found to be very wide and of variable morphology between corn snakes, as has been reported in other reptile species. This segment was approximately two to three times longer than the QT of mammals but, similar to mammals, was around twice the duration of the PR (PQ) interval. High numbers of snakes demonstrated PR depression followed by a curvilinear elevation from the end of the R (J-point) to the T wave, which caused difficulties in establishing the isoelectric baseline along traditional mammalian lines. This prompted the creation of a new baseline at the early part of the TP segment (figure 4) which was consistently more level. In human cardiology, PQ elevation and ST depression, either separately or concurrently, are clinically significant and associated with myocardial infarction and myocarditis. Given the high number of subjects in the present study demonstrating this feature and the general good health of the cohort, these segmental fluctuations are unlikely to have the same pathological importance in corn snakes, although the authors could speculate that PR elevation and ST depression indicate a normal degree of myocardial ischaemia in the heart of the snake. The embryonic hearts of birds and mammals demonstrate a great inherent tolerance to ischaemia, and as the hearts of adult reptiles are structurally very similar it is possible that this is a shared characteristic. An alternative explanation could be mechanical and related to the relatively unique anatomy of the cardiac region in snakes. The attachments of the ophidian heart in the cranial coelom are loose when compared with their mammalian counterparts and allow for the passage of whole prey items. They could permit a rhythmic swinging of the heart through the contraction cycle, leading to a regular oscillation of the ECG baseline. This could be investigated further by recording an ECG, using the arrangement of ECG pads herein described, while simultaneously performing echocardiography.

| Table 2 | Weight, length, HR and MEA for group and sex (male and female) |
|---------|---------------------------------------------------------------|
| Parameter | Group (n=29), mean±sd | Female (n=10), mean±sd | Male (n=19), mean±sd |
| Weight (g) | 609±216 | 583±189 | 622±233 |
| Length (cm) | 114±24 | 117±25 | 113±24 |
| HR (bpm) | 60±18 | 53±17 | 65±18 |
| MEA (°) | 66±12 | 64±13 | 70±11 |

bpm, beats per minute; HR, heart rate; MEA, mean electrical axis.
The HRs that the authors obtained are in general agreement with those reported in other ophidian studies and are not dissimilar from other representatives of the class Reptilia (tables 3 and 4). They are however much slower than mammals and reflect the approximately 10-fold lower metabolic rate seen in reptiles. The HR of snakes can be affected by a wide range of intrinsic and extrinsic factors, but in general it is increased with elevated body temperature, small body size, external stimuli (such as handling), recent meal ingestion and gravidity. Previous studies involving ECG in snakes have been highly variable in design and have frequently included data from small cohort numbers, or large numbers of variable in design and have frequently included data from small cohort numbers, or large numbers of species restrained by different physical and chemical methods. The data in the present study, obtained from only one species, came from a larger study cohort, and the authors’ protocol minimised confounders. Nonetheless, these data resemble closely those obtained from studies where confounding factors have not been so rigorously controlled, suggesting that they may have less influence on HR and cardiac conductivity than previously supposed.

Typically in mammals, as HR increases, the period of time between the T wave and the P wave of the next cardiac cycle shortens. In the study here reported, shortening of the TP interval was also observed in snakes with HRs at the upper end of the population range. In some cases this shortening was so extreme that there was merging of T and P waves. Germer et al. mentioned T and P wave merging in a group of geckos, while Mullen and Jacob and McDonald described wave overlap at the TP interval or ‘masking’ of the P wave by the T wave in cohort subgroups of wild caught snakes. These authors do not speculate on the possible causes of this finding, but Clarke and Marx identified handling stress as an important cause of increased HRs in wild caught snakes. In the present study, while all possible efforts were made to reduce stress at the time of ECG measurement and although all individuals were captive bred pet animals, it was clear that some snakes were initially wary of interference and took greater time to settle. This group of snakes tended to have higher HRs and more pronounced TP shortening. The authors conclude that corn snakes have an inherent variability to handling tolerance, but also and more importantly that marked shortening of the TP segment or TP merging may be an additional useful, quantifiable indicator of stress not only for captive corn snakes but for snakes in general, a collection of animals for which behavioural indicators of stress can be very vague. The physiological advantage of this response is not clear. Atrial depolarisation so close to ventricular repolarisation in snakes could significantly decrease time available for passive ventricular filling (which predominates in mammals), even at HRs considered low in mammals, increasing reliance on the atrial contribution to ventricular diastole. If confirmed by, for example, spectral and tissue Doppler echocardiography, it would suggest a profound difference in the mechanics of diastole in corn snakes compared with those in turtles and pythons, for which ventricular suction is thought to play an important role.

The authors also investigated whether sex might have an influence on HR. Follicular genesis can occur in female snakes independent of the presence of males and sporadically throughout a captive snake’s life. It would be reasonable to expect that the elevated metabolic rate associated with this condition would lead to an increase in HR. The authors did not attempt to determine the level of ovarian activity in individual females through an additional ultrasound examination due to concern of increased confounding stress effects. However, they did not identify a significant increase in HR in the female corn snake population when compared with males. Other factors such as weight and snout-to-vent length were also found to have no significant relationship with HR. Therefore, the authors believe that the range of HR that was recorded in both male and female corn snakes is representative of the general corn snake population.

The MEA in reptiles has historically been regarded as very challenging (if not impossible) to determine and of limited clinical value. By contrast, in the study here reported, identification and measurement of R waves on leads 1 and 3 were readily performed and the MEA was easily calculated. Importantly, the technique the authors described produces MEA through electrode placement and draws similarities to companion mammals, rather than MEA as defined by cardiac alignment and physical measurements. Yielded values demonstrated a left axis dominance (table 2), indicating that it is the left side of the common ventricle in corn snakes that makes up most of the myocardium. This axial dominance is similar to mammals, where the left ventricle is larger than the right, but is also similar to the few other reptile species for which MEA has been studied.

### Table 3 Snake heart rate ranges by study

| Study                   | Heart rate range (beats per minute) |
|-------------------------|-------------------------------------|
| Clarke and Marx          | 9–72                                |
| Mullen                  | 22–136                              |
| Valentinuzzi et al      | 13–62                               |
| Anderson et al          | 70–73                               |
| Martyn G Lewis, Jonathan Bouvard, Kevin Eatwell, Geoff Culshaw, unpublished data | 42–78 |

### Table 4 Reptile heart rates: study, species and range

| Study                   | Species                          | Heart rate range (beats per minute) |
|-------------------------|----------------------------------|-------------------------------------|
| Heaton-Jones and King   | American alligator               | 31–42                              |
| HoZ and HoZ             | Red-eared slider                 | 16–36                              |
| Martinez-Silvestre et al | Gomera giant lizard          | 35–60                              |
| Tan et al               | Marbled water monitor            | 26–58                              |
| Hunt                   | Central bearded dragon           | 24–170                             |
calculated, indicating a common asymmetrical distribution of ventricular muscle mass in reptiles. The authors also found that MEA values produced in this study were highly conserved between individuals, suggesting a predictable direction of electrical flow through the myocardium and therefore a broadly uniform positioning of the heart within the coelom. The authors suggest that the ECG in corn snakes generates a vector of MEA that is quantifiable and has the potential to aid in the diagnosis of cardiac disease in snakes, similar to its use in mammals, and further that the technique described herein will permit the calculation of MEA to the benefit of future ophidian studies.

**Limitations**

There were a number of limitations to this study. First, the exact ages of the snakes involved was not always known. Size (in terms of snout-to-vent length and weight), which was used to classify individuals as adult, may be inaccurate as growth can be influenced by a number of factors, including sex, long-term nutrition and captive environment. However, known adult animals were present in the study population and no difference in bodyweight or length measurements was identified between male and female snakes. It is therefore reasonable to consider the cohort as homogeneous in terms of size and, by extension, adult age. Secondly, despite a comprehensive clinical examination, the absence of cardiac or non-cardiac disease could not be excluded from the study group. Inclusion of routine blood sampling and comprehensive echocardiography would have helped to identify systemic disease and structural or functional cardiac disease, respectively. These investigations, however, fell outside the scope of the study hypothesis and may have introduced a significant confounding stress effect on the data. Furthermore, the limits of ethical approval precluded invasive procedures (such as blood sampling), or undue stress through prolonged or repeated handling. Thirdly, the cohort size was insufficient to provide reference ranges for this species. However, to the authors’ knowledge, this is the largest single-species ophidian study that has been published.

Finally, there were twice as many males as females in this study, which could have introduced sex bias that was not identifiable on the variables that were measured. However, excluding females from analysis would have reduced the statistical power of the study.

**Conclusion**

As hypothesised, ECG waveforms in corn snakes are morphologically recognisable and can be characterised and interpreted in a similar way to ECGs obtained from mammals. However, there are important differences, such as HR and particularly TP intervals, that may be useful in identifying corn snakes experiencing stress. The data presented here could help provide reference ranges for ECG parameters in this species, aiding the clinical application of ECGs in general and specialist practice.

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**Competing interests**

None declared.

**Ethics approval**

Ethical approval was obtained from an independent Edinburgh University Veterinary Ethical Review Committee (VERC).

**Data availability statement**

No data are available.

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