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Electrocardiographic findings in anesthetised ferrets (*Mustela putorius furo*) of different ages

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A R T I C L E I N F O

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A B S T R A C T

Ferrets are an emergent companion animal species, and a possible animal model for conditions such as COVID-19 infection. As such, they are more present in veterinary practice than in the past. This study aimed at assessing how ferrets' electrocardiographic parameters varied according to the age of the animal during isoflurane anesthesia. Eighteen ferrets, divided into three age categories (3–6 months: 7 ferrets; 32–36 months: 4 ferrets; >55 months: 7 ferrets), were included in the study. Generalized Estimation Equations, with Bonferroni corrected pairwise comparisons for age categories, were run. Most of the measured electrocardiographic parameters varied depending on age (all $p < 0.05$), although their trend across the three age groups was not the same for all the parameters. S wave amplitude and P wave duration varied according to sex. Findings of the present study can be useful for veterinary practitioners dealing with ferrets.

Ferrets' popularity as companion animals increased the last decades (Bradley Bays et al., 2006; Zandvliet, 2005), leading to an enhanced need for veterinary expertise on this species (Bublot et al., 2006), especially regarding cardiology, as cardiac problems are relatively common in middle-aged to older ferrets (van Zeeland and Schoemaker, 2022).

The ferret is considered also an important experimental species, in particular following the outburst of COVID-19, because they represent an animal model that may facilitate studying the efficacy of SARS-CoV-2 therapeutics and vaccines (Au et al., 2022; Kim et al., 2020). In this respect it is noteworthy that cardiac injury is one of the possible effects of the virus both in humans and dogs (Romito et al., 2021).

Although some study targeting ferrets' electrocardiographic (ECG) parameters have already been performed (Bone et al., 1988; Smith and Bishop, 1985; Dudás-Györki et al., 2011; Malakoff et al., 2012), differences due to age in ferrets' ECG parameters have not been fully investigated yet, with the partial exception of Bublot et al.'s study (Bublot et al., 2006).

In humans, age and sex dependent variations linked to heart weight and body mass (McFarlane, 1065), or to physiologic changes of the cardiac structure due to aging have been described (Murpy et al., 2022). Similar ECG age and sex dependent variations have been linked to heart weight and body mass. Physiologic changes to the cardiac structure secondary to aging have also been described in other animals, such as dogs, Guinea pigs, rabbits, and mice (Murpy et al., 2022).

The aim of the present study was to assess whether and, in case, how, ferrets' ECG parameters varied according to age and sex of the animal during isoflurane anesthesia.

The clinical data used in this work were collected during routine clinical procedures involving ferrets and were not from procedures performed for experimental purposes. Before the ECG procedure, animals underwent a thorough physical examination, including a detailed examination of the cardiovascular and the respiratory systems, and their clinical history was recorded.

Eighteen clinically healthy animals were included in the study and grouped into three different age categories: Young (Y, 2–6 months of age, N = 6; 3 females, 3 males), Young Adult (YA, 32–36 months of age, N = 4; 2 females, 2 males) and Old Adult (OA, > 55 months of age, N = 8; 5 females, 3 males). No testing for COVID-19 was done, because the study took place before the COVID-19 outbreak.

After physical examination, the animals were anesthetized using isoflurane administered via a respiratory mask. Induction was performed using O_2 100 % at 2–3 L/minute, with 5 % isoflurane (Isoflo, Abbott Laboratories ltd., Maidenhead, UK) for 60–90 s. After that time, the anesthetic flow was reduced to 1.5 % - 2% for the time needed for

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electrocardiographic parameters recording (i.e., approximately one minute). Then the anesthetic administration was discontinued, while the O_2 100 % at 2–3 L/minute flow was maintained for some seconds (maximum 40″) in order to facilitate recovery. Overall, ferrets were anesthetized for a maximum of 3 min. We did not monitor SpO2 during the procedure because it was of short duration and minimally invasive.

The ECG trace was recorded using a commercially available electrocardiographic machine (ESAOTE P80, ESAOTE S. p. A., Genova, Italy). The ECG machine was placed on a shower plastic mat positioned over an examination table. Each ferret was positioned in right lateral recumbency, with legs perpendicular to the longitudinal axis of the body and not in contact among them. Manipulation was kept to a minimum during recording in order to minimize possible interferences. Electrodes were positioned on the proximal third of the limbs, on the caudal margin of the elbow, and on the cranial margin of the knee. Alcohol was applied on each electrode and the corresponding skin fold to insure optimal electric conductivity. Both the automatic and the manual recording modes of the machine were used. The former recorded around 11 complete complexes for each bipolar and each unipolar lead with a paper speed = 50 mm/s and a standard sensitivity of 1 cm/1mV, whereas during the latter, the paper speed was decreased to 25 mm/s and 10 mm/s and the sensitivity varied form 0.5 cm/1mV to 2 cm/1mV depending on the amplitude of the trace.

We used the first five regular P-QRS-T complexes recorded on lead II without muscular interferences to calculate the electrocardiographic parameters detailed in Table 1. Because not all the complexes were at the same level, we used a mean baseline line calculated as the mean of the beginning of the P wave of the used five complexes to calculate the amplitude of the deflections on the electrocardiographic trace.

MEA was calculated following the equation:

$$a^\circ = \mathrm{arcTg}[(OA + 2OB)/Tg60^\circ * OA]$$

where a° stands for the direction of the main cardiac electric axis, OA for the algebraic sum of QR in lead I and OB for the algebraic sum of QR in lead III. Heart rate (HR) was calculated around 2 min from the initial restraint of the animal (T0) and 21 s after T0 (T1).

Heart rates at T0 and T1 were compared using a Student T test. The electrocardiographic variables considered in this study (Table 1) were analyzed by the Generalized Estimating Equations (GEEs) procedure, with individual as subject, sex (females vs males) and age (Y vs YA vs OA) as independent variables, and Bonferroni corrected pairwise comparisons among age groups (alpha = 0.05). Statistical analyses were performed using the SPSS software (SPSS ver. 27, IBM, Armonk, NY, USA).

All ferrets showed a normal sinus rhythm for this species (Bublot et al., 2006; Dudás-Györki et al., 2011) without the presence of atrioventricular block which has been reported to be a common condition in these animals (Malakoff et al., 2012). Electrocardiographic recordings were normal (Bone et al., 1988) and all animals were clinically healthy. Few additional qualitative modifications of the electrocardiographic tracings were observed including S-T elevation, deep S wave and splintered QRS complex in one ferret each.

In this study, HR (Table 1) was lower than that observed by (Bublot et al., 2006), in ferrets anesthetized with a combination of ketamine and diazepam. Indeed, ketamine increases HR, whereas isoflurane decreases it (Cantwell, 2001). The hypothesis of a depressive effect of isoflurane on HR agrees with the HR reduction between T0 and T1, found especially in Y and OA ferrets (Table 1). However, the HR found in this study falls within the normal range (210–405 bpm) for the species, found in conscious ferret by Dudás-Györki et al. (Dudás-Györki et al., 2011) suggesting that the used anesthetic protocol did not significantly depress cardiac activity.

The effects of age and sex on electrocardiographic parameters are reported in Table 2. Age affected HR, which was significantly lower in OA than Y ferrets at T0 ($P < 0.05$), and R wave amplitude, which was higher in OA than Y ferrets ($P < 0.001$). These findings agree with those by (Bublot et al., 2006), who compared the same parameters in a group of 80 ferrets, whose age varied from 2 to 72 months. The QRS complex amplitude was higher ($P < 0.001$) in both A and YA than Y animals, and this was probably dependent on the differences observed in the R wave amplitude. The S wave amplitude was significantly lower ($P < 0.01$) in Y than in YA subjects, and this disagrees with the findings of (Bublot et al., 2006) who did not find any age-dependent difference in the S wave amplitude. On the other hand, no age-related differences in P wave amplitude and duration, Q wave amplitude, PR interval and MEA were found in this study, in agreement with (Bublot et al., 2006). The RR interval was significantly ($P < 0.05$) shorter in Y than OA subjects and was obviously related to HR. Interestingly, no age related difference in the QRS complex

Table 1
Electrocardiographic variables in the18 anesthetised ferrets.

Variable	Unit	Definition	Mean Y	Mean YA	Mean OA
HR T0	bpm	Heart rate at T0	242.0	237.3	222.7
HR T1	bpm	Heart rate at T0	224.4	229.0	215.3
RR#	ms	RR interval duration	238.1	257.7	274.6
Ps#	ms	P wave duration	23.6	26.5	23.6
P#	mV	P wave amplitude	0.1	0.1	0.1
Q#	mV	Q wave amplitude	0.0	0.1	0.0
R#	mV	R wave amplitude	1.6	2.3	2.9
S#	mV	S wave amplitude	0.0	0.1	0.1
T#	mV	T wave amplitude	0.2	0.2	0.2
PR#	ms	Time interval between the start of the P wave and the end of the Q wave	44.0	28.8	42.7
QRSs#	ms	Voltage difference (amplitude) between the maximum value of the R wave and the maximum negative deflection (which could be either in the Q or the S wave)	39.1	38.6	35.3
QRS#	mV	Time interval between the start of the Q wave and the end of the S wave	1.6	2.5	3.0
QT#	ms	Time interval between the start of the Q wave and the end of the T wave	119.0	150.0	148.1
QTc#	ms	Time interval between the start of the Q wave and the end of the T wave corrected for heart rate: $QTc = QT^* \sqrt{1/RR}$	7.7	9.4	9.0
QR lead I	mV	Voltage difference (amplitude) between the maximum value of the R wave and the maximum negative deflection in the Q wave on the first lead	0.1	0.0	0.4
QRD lead III	mV	Voltage difference (amplitude) between the maximum value of the R wave and the maximum negative deflection in the Q wave on the third lead	1.6	2.0	2.7
MEA	°	Cardiac axis	36.5	46.0	84.6

T0 = around 2 min from the initial restraint of the animal, T1 = 21 s after T0. Y: Young, YA: Young Adult, OA: Old Adult. #Unless otherwise specified, the variables were recorded on lead II.

Table 2
Outcome of Generalized Estimating Equations (GEEs; Wald Chi-square statistic) and medians (min–max) of the electrocardiographic variables observed. Y: Young, YA: Young Adult, OA: Old Adult; F: female, M: male. Level of significance: *** $P > 0.001$; ** $P < 0.01$; * $P < 0.05$. Different superscripts in the same row indicates a statistically significant differences between age groups ([a,b,c] $P < 0.05$).

Variable	Intercept (df = 2)	Age (df = 2)				Sex (df = 1)		
	Wald chi-square	Wald chi-square	Y (N = 6)	YA (N = 4)	OA (N = 8)	Wald chi-square	Female (N = 10)	Male (N = 8)
HR T0	1171.500***	6.300*	244[a] (210–263)	239[ab] (188–283)	219[b] (205–261)	4.260*	218.5 (188–268)	251.5 (210–283)
HR T1	1068.030***	1.422	233 (203–248)	236 (169–275)	211 (192–256)	4.907*	207 (169–259)	233.5 (211–275)
RR	972.446***	7.593*	250[a] (184–280)	252.5[ab] (210–320)	280[b] (220–310)	1.647	275 (184–320)	247.5 (210–290)
Ps	1106.574***	2.491	25 (20–35)	25 (20–40)	25 (20–30)	6.471*	25 (20–40)	20 (20–30)
P	257.446***	5.991	0.1 (0.05–0.15)	0.15 (0.10–0.20)	0.15 (0.05–0.25)	1.028	0.105 (0.05–0.2)	0.1 (0.0–0.25)
Q	10.435**	2.267	0.02 (0.0–0.5)	0.06 (0.0–0.35)	0.02 (0.00–0.10)	0.749	0.02 (0.00–0.5)	0.05 (0.00–0.35)
R	236.856***	19.752***	1.5[a] (1.15–2.71)	2.525[ab] (1.2–3.05)	3.0[b] (2.00–4.30)	1.437	2.4 (1.15–4.3)	1.8 (1.2–3.2)
S	11.455***	11.514**	0.00[a] (0.00–0.02)	0.125[b] (0.00–0.25)	0.02[ab] (0.00–0.70)	5.244*	0.00 (0.00–0.1)	0.1 (0.00–0.7)
T	78.065***	0.404	0.25 (0.05–0.4)	0.18 (0.05–0.3)	0.18 (0.00–0.40)	0.070	0.25 (0.00–0.4)	0.165 (0.05–0.4)
PR	1234.757***	36.509***	45 (35–50)	30 (20–40)	45 (25–50)	2.102	40 (20–50)	45 (20–50)
QRSs	527.852***	0.725	40 (30–45)	40 (30–50)	35 (20–60)	0.491	35 (20–45)	40 (20–60)
QRS	240.740***	20.804***	1.52[a] (1.17–2.75)	2.575[b] (1.45–3.35)	3.02[b] (2.07–4.40)	0.818	2.45 (1.17–4.4)	1.86 (1.3–3.4)
QT	657.749***	9.446**	110[a] (95–160)	150[b] (120–190)	140[b] (110–200)	0.033	135 (100–180)	125 (95–200)
QTc	834.192***	8.487*	7.16[a] (6.32–9.58)	9.15[b] (8.28–11.27)	8.22[b] (6.69–13.48)	0.312	8.38 (6.32–10.22)	8.13 (6.40–13,48)
QR lead I	4.386*	10.298**	0.15[a] (-0.07–0.35)	0.25[ab] (-1.05–0.44)	0.4[b] (0.2–1.0)	0.305	0.25 (-1.05–1.0)	0.3 (-0.07–0.45)
QRD lead III	157.246***	7.976*	1.4[a] (1.1–2.9)	2.3[ab] (0.9–2.7)	2.4[b] (1.4–4.2)	1.609	2.15 (1.25–3.8)	1.4 (0.9–4.2)
MEA	15.184***	3.947	86.14 (-89.52–89.80)	83.22 (-72–25-87.70)	85.01 (78.07–87.76)	0.163	85.54 (-89.52–89.8)	84.81 (-88.56–87.76)

duration was observed, while the QT segment was shorter in Y than in both YA and OA subjects ($P < 0.01$), even when corrected for HR (OTc, $P < 0.05$).

Sex-related differences were found in HR, S wave amplitude and P wave duration. In particular, HR was lower in females than in males at both T0 and T1 ($P < 0.05$), differing from the findings of (Bublot et al., 2006; Dudás-Györki et al., 2011), who found higher hearth rates in female than male conscious ferrets. S wave amplitude was higher in males than females, whereas the P wave lasted longer in females than males. Such differences were not found in (Dudás-Györki et al., 2011). Caution should be used to generalize the results of the present study to conscious ferrets, because changes of ECG parameters have been described in isoflurane-anesthetized animals (Haq et al., 2022; Svork and Svork, 2022).

In conclusion, this study identified an age effect in the amplitude of the R and S waves and QRS complex. The effect on the S wave, to our knowledge, has never been reported before. In addition, sex related differences were observed.

Despite the interest about the ferret as a companion and, potentially, experimental animal, information about the cardiac function in this species is scarce, making the findings of this study useful for veterinarians. However, the small number of subjects, in particular in the YA group, suggests some caution and should stimulate further investigation.

Ethical statement

Clinical observations and biological samples used in this work were collected during routinely clinical procedures not performed for experimental purposes. According to the Italian law for the protection of experimental animals (Law Decree n. 26, 4 March 2014, art. 2), the approval by an ethical committee is not required under the circumstances that this trial was performed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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