



# ELECTROCARDIOGRAPHIC REFERENCE VALUES FOR HEALTHY NETHERLAND DWARF RABBITS AND THE INFLUENCE OF BODY POSITION. AGE AND GENDER

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Abstract: The aim of this study was to provide reference values for a single, popular breed of pet rabbit. Moreover, additional objectives were to determine whether sex, body position or age alter Netherland Dwarf rabbit electrocardiographic variables and whether the use of electrocardiographic filters affects those variables. Forty Netherland Dwarf rabbits were examined clinically and standard six-lead electrocardiograms (ECGs) were recorded in sternal and then dorsal recumbency. At first power-line and anti-drift filters were used and then they were disabled. The following variables were measured in lead II: heart rate; P wave duration and amplitude; P-R interval; QRS duration; R wave amplitude (with and without filters); Q-T interval; T wave duration and amplitude; S-T segment; J-T duration; and mean electrical axis (MEA) (with and without filters). MEA was determined by 3 different methods. After statistical processing of the data, our results showed that there were no significant differences between both recumbencies, with the exception of the J-T duration, which was higher in dorsal recumbency. The R wave amplitude using electrocardiographic filters showed significant differences between males (0.083 mV) and females (0.115 mV; P<0.05); and between younger rabbits (0.108 mV) and older rabbits (0.097 mV; P<0.05). These differences were not shown between R waves with filters disabled. Moreover, the strongest correlation was between 2 MEA methods without filters. MEA was more leftward in the pet rabbit than in other species (dog or cats). In conclusion, electrocardiography recording without electrocardiographic filters should be assessed when it is possible, and the specific ECGs characteristics for Netherland Dwarf rabbit should be taken into account.

Key Words: electrocardiography, heart, filters, mean electrical axis, rabbit.

### INTRODUCTION

Rabbits (Oryctolagus cuniculus) have been used for many years as farm animals, experimental animals, and most recently as pets. The Netherland Dwarf breed is one of the most common breeds kept as pets. In our experience, not all those clinical physiological variables (e.g., packed cell volume, urea...) applied to farm or lab animals can be applied to pet rabbits, due to different environmental, breed and management conditions that determine peculiar pathological processes.

Nowadays, knowledge about the incidence, diagnosis and treatment of cardiac diseases in pet rabbits is lacking, although cardiomyopathy is a frequent finding in older or laboratory rabbits (Pariaut, 2009; Lennox, 2010; Lord et al., 2010). In cardiac diseases, electrocardiography is an essential technique to determine heart status. To this end. electrocardiogram should be taken in animals with tachycardia, bradycardia or arrhythmia, acute onset of dyspnoea, cardiac murmurs or shock (Tilley, 1985). Several publications about this technique in the rabbit have appeared in journals. However, almost all of these research works have reported electrocardiogram in the rabbit as experimental animal (Lieberman et al., 1954; Saitanov, 1960; Szabuniewicz et al., 1971; Meral et al., 1998; Farkas et al., 2004).

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In many of these articles, steel needles were injected subcutaneously as electrodes, but alligator clips are commonly used in small animal practice (Tilley, 1985; Edwards, 1987; Martin, 2001), Owing to the fact that rabbits have a thin skin, toothless alligator clips should be used (Turner Giannico et al., 2015) or with the teeth bent outwards (Lord et al., 2010).

Among the different methods of handling and restraining rabbits, the trancing technique has been described (Ward. 2006; Varga, 2014). Hypnosis/trancing is a useful technique to carry out non-painful procedures, such as examination of the abdomen, genitalia and feet, nail clipping or abdominal ultrasound/radiography (Malley, 2007; Varga, 2014; Chapel et al., 2015). However, this method is not appropriate for rabbits with breathing difficulties (Malley, 2007).

Although several electrocardiographic variables have been reported (Saitanov, 1960; Szabuniewicz et al., 1971; Meral et al., 1998; Fraser and Girling, 2009; Pariaut, 2009; Lord et al., 2010; Turner Giannico et al., 2015), the mean electrical axis (MEA) in the frontal plane showed variability in the values and ranges between the different reports. Moreover, to the authors' knowledge no studies of electrocardiogram in "trancing" rabbits (dorsal recumbency) have been reported, with the exception of Saitanov (1960), showing that no significant differences were found between supine and prone positions. Recently, Rishniw et al. (2002) found that R wave amplitude and MEA were changed depending on position in dogs. These findings were also observed in cats by Harvey et al. (2005).

Different methods and formulas can be used to determine the MEA. According to Novosel et al. (1999), MEA was different depending on the formula used.

On the other hand, the use of electrocardiographic filters causes an attenuation in wave amplitudes (Martin, 2001). Schrope et al. (1995) reported a significant effect of R wave amplitudes when frequency filters were used in cats. Furthermore, Luo and Johnston (2010) published that T-P segment changes could be caused by a high-pass filter when a linear phase at higher cut-off (0.67 Hz) is used and when the heart rate is very low.

Taking into account the above mentioned information, the aim of our study was to provide reference values and to evaluate the effect of body position, sex and age on the electrocardiogram in normal Netherland Dwarf rabbits without anaesthetic or sedation. In addition, 2 main objectives were proposed: 1) to determine whether electrocardiographic filter settings affect R wave amplitude and/or MEA; 2) to assess the differences between the 3 methods used for measuring the MEA.

### MATERIALS AND METHODS

#### Animals

Privately owned Netherland Dwarf rabbits (n=44) were recruited voluntarily through contacts with owners. Twentyeight rabbits were intact female, 10 were intact males and 6 were neutered males. Rabbits had a mean age of 12.5 mo (range: 9-19) and a mean weight of 1.39±0.24 kg. Before recording electrocardiograms (ECGs), all rabbits were examined to confirm that they did not have any respiratory and cardiac disease, through clinical assessment, auscultation, arterial blood pressure and a simple blood test profile including the evaluation of blood glucose, urea, electrolyte profile (sodium, calcium and phosphorus) and acid-base balance.

### Electrocardiographic analysis

All the rabbits were manually restrained without using sedation or anaesthetic. They had ECGs recorded in 2 different recumbencies; first, sternal recumbency (ST) and then dorsal recumbency (DR) with "trancing" method (Ward, 2006; Chapel et al., 2015). Toothless alligator clip electrodes were used to avoid injuries to rabbit skin and were applied cranially at the triceps brachii muscle (lateral head) in both forelimbs to avoid respiratory movements (Martin, 2001) and craniadistally at the biceps femoris muscle; these locations were similar to those that had been used by Meral et al. (1998). Moreover, the hair in those positions was clipped and contact gel (Melabine®, Bastos Medical S.L., Spain) was used to enhance conduction. Rabbits were allowed some time to become relaxed and then ECGs of 6 leads (I, II, III, aVR, aVL and aVF) were recorded using a CARDIETTE® start 200 hv (H&C Medical Devices SPA, Italy) electrocardiograph. ECGs were printed at 25 and 50 mm/s, and 20 mm/mV for at least 6 s (Figure 1). At first, power-

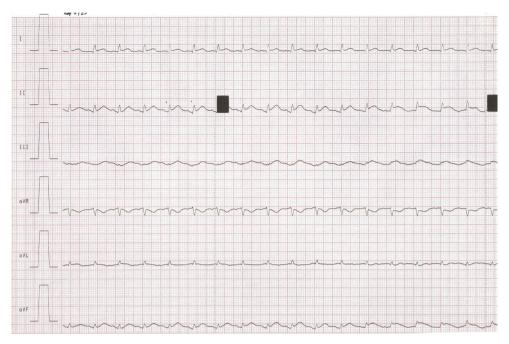


Figure 1: Example of an electrocardiogram of a female Netherland Dwarf rabbit, 18 mo old, recorded at 20 mm/mV and 50 mm/s. Leads L - III and aVR - aVF are shown.

line filter (modified 50 Hz notch digital filter) and anti-drift filter (0.5 Hz pass high and linear phase digital filter) were used; after that, these filters were removed in order to compare R wave amplitude and MEA with and without filters.

Variables measured manually on printed ECG in lead II at 50 mm/s were averaged from 3 cycles. These variables were: heart rate and rhythm (at 25 and 50 mm/s; using 6 s, and multiply number of QRS complexes by 10); P wave duration and amplitude; P-R interval; QRS duration; R wave amplitude (with and without filter); Q-T interval; T wave duration and amplitude; S-T segment; J-T duration; and MEA (with and without filter). The Q-T interval was measured according to Farkas et al. (2004). Furthermore, MEA was determined by Tilley's (1985) method [MEA1], and it was also measured by the sum of the same amplitudes in lead I and aVF and calculated by the equations proposed by Novosel et al. (1999) and Hamm and Willems (2010):

MEA2=±arctangent (aVF/I)

MEA3=±arctangent (2aVF/√3I)

#### Statistical methods

All the data obtained were analysed using the statistical program SPSS v.20 for Windows (IBM). Different ECG variables were tested for normal distribution using the Shapiro-Wilk test. Those variables not normally distributed were transformed into normal distribution using a logarithmic transformation. All the variables were measured in 2 different body positions (ST and DR), and distributed taking into account sex (male and female) and age range (< or ≥12 mo). Results were analysed using a Univariate General Linear Model, and means between groups were compared by a paired t-test. Regarding R waves and MEAs, these were measured with and without filters and differences between groups were estimated using a paired t-test. Also, Pearson's correlation and a polynomial regression analysis between R wave amplitudes (with and without filters) and body weight data were performed. Statistically significant differences were accepted if P < 0.05.

### **RESULTS**

Four rabbits (2 male and 2 female) had to be rejected because they showed evidence of right bundle-branch block (RBBB) in the electrocardiogram (QRS duration incremented and wide S wave), although they had not shown clinical signs. The remaining rabbits showed a regular sinus rhythm without differences over 0.04 s (2 mm) in several R-R intervals. The S-T segment was isoelectric or presented a slight elevation in all the electrocardiograms. Moreover, the following QRS complex patterns have been found in lead II in our research: qR, R, Rs, rs and RS. The Rs pattern was the most frequent in lead II regardless of body position, sex or age; followed by the R and gR patterns. We also found spontaneous changes in QRS pattern between the sternal and dorsal position in 2 rabbits aged less than 12 mo. The pattern changed from RS and rs in sternal recumbency to Rs and gR in dorsal recumbency, respectively.

The results obtained from the different ECG variables are shown in Table 1. There was no significant difference in the interaction of the 3 fixed factors, so P-value is expressed independently for each fixed factor (body position, age and sex) in Table 1.

## R wave amplitude: effects of filters

According to our first objective, the R wave amplitude with and without filters was significantly different (P<0.001) (see Figure 2), so the R wave amplitude without filters was larger (0.270±0.043 mV vs. 0.102±0.082 mV) than with them and the ECG tracing obtained with the use of filters is clearer and free of artefacts. Besides, the strength of association between both measurements was slightly high (r=0.653) and the correlation coefficient was very significantly high (P<0.001), i.e. the 2 increased their values proportionally.

On other hand, Pearson's correlation showed a moderate strength of association between body weight and R wave with filters (r=0.411; P=0.004). Nevertheless, a polynomial regression did not identify any relationship between them.

Table 1: Means and standard deviation of electrocardiographic variables in 40 Netherland Dwarf rabbits measured from lead II with the exception of mean electrical axis.

	Body position		Age (months)		Sex		P-value		
	ST (n=40)	DR (n=40)	<12 (n=26)	≥12 (n=14)	Male (n=14)	Female (n=26)	Pos.	Age	Sex
Hr25 (bpm)	218±26.5	204±27.0	195±22.5	234±28.0	206±24.5	210±26.0	NS	0.014	NS
Hr50 (bpm)	224±25.0	211±27.0	202±22.0	239±28.0	213±24.0	216±26.0	NS	0.015	NS
P-D (s)	0.026±0.005	0.027±0.005	0.027±0.004	0.025±0.005	0.028±0.004	0.026±0.003	NS	NS	NS
P-A (mV)	0.039±0.014	0.037±0.010	0.040±0.012	0.035±0.011	0.038±0.003	$0.038 \pm 0.006$	NS	NS	NS
PR-I (s)	0.054±0.007	0.053±0.008	0.056±0.006	0.049±0.009	0.053±0.010	0.054±0.006	NS	0.006	NS
QRS-D (s)	0.039±0.006	0.036±0.004	0.038±0.005	0.038±0.004	0.038±0.003	$0.038 \pm 0.002$	NS	NS	NS
R-awf (mV)	0.113±0.040	0.092±0.043	0.108±0.034	0.097±0.045	0.083±0.025	0.115±0.036	NS	0.033	0.012
R-anf (mV)	0.294±0.060	0.247±0.089	0.286±0.078	0.24±0.086	$0.269 \pm 0.071$	0.271±0.089	NS	NS	NS
QT-I (s)	0.145±0.018	0.151±0.016	0.154±0.015	0.138±0.014	0.151±0.016	0.147±0.017	NS	0.002	NS
T-D (s)	0.066±0.014	0.072±0.014	0.075±0.012	0.062±0.014	$0.071 \pm 0.011$	0.072±0.009	NS	0.015	NS
T-A (mV)	0.071±0.022	0.075±0.029	0.082±0.027	0.065±0.019	0.083±0.023	0.072±0.026	NS	NS	NS
J-T D (s)	0.035±0.008	0.040±0.009	0.038±0.010	0.032±0.007	0.036±0.009	0.036±0.008	0.03	0.029	NS
MEA <sup>a</sup>	1±43	16±39	3±37	20±48	-19±33	23±39	NS	NS	NS_

ST: sternal recumbency; DR: dorsal recumbency; Hr25: heart rate at 25 mm/s; Hr50: heart rate at 50 mm/s; bpm: beats per minute; D: duration; A: amplitude; I: interval; awf: amplitude with electrocardiographic filters; anf: amplitude with filters disabled; MEA: mean electrical axis.

adetermined by Tilley's technique (1985); NS: not significant.

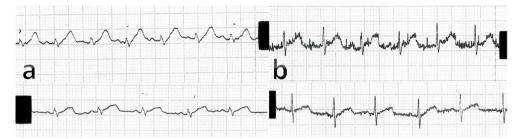


Figure 2: Lead II in 2 electrocardiographic tracings from 2 Netherland Dwarf rabbits with the use of filters (a) and with them disabled (b).

#### MEAs assessment

The values obtained from the different MEAs with the use (or not) of filters are shown in Figure 3. There were no significant differences between MEAs and their relationship is expressed in Table 2. The strongest correlation was observed between MEA1 and MEA2 without electrocardiographic filters (r=0.931).

## Comparison between sternal and dorsal body position

All the rabbits (n=40) were compared according to their body position. There was only one significant difference in body position between groups in relation to J-T segment duration (P<0.05), showing that its mean value was higher in sternal position (0.040 s) than in dorsal position (0.035 s).

## Comparison by age

For this objective, 26 rabbits aged less than 12 mo (mean: 10.2) were compared to 14 rabbits with an age higher than 12 mo (mean: 17.8). Younger rabbits had a lower heart rate than older rabbits at both speeds, 25 mm/s (P=0.014)

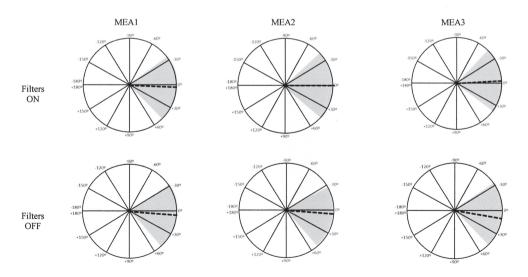


Figure 3: Mean electrical axis (MEA) range (interval confidence 95%; shaded) and mean (dotted line) for the 3 techniques and with the use (or not) of electrocardiographic filters. MEAs were determined by Tilley's (1985) method [MEA1],  $\pm$ arctan (aVF/I) [MEA2] and  $\pm$ arctan(2aVF/ $\sqrt{3}$ I) [MEA3].

Table 2: Pearson's correlation coefficients between the different methods to calculate the mean electrical axis (MEA).

MEA1W	0.815***	0.895***	_	0.650***	_
	MEA1Wout	_	0.931***	_	0.614***
		MEA2W	0.774***	0.749***	_
			MEA2Wout		0.671***
				MEA3W	0.782***
					MEA3Wout

W: with electrocardiographic filters: Wout: without electrocardiographic filters. \*\*\*: P<0.000.

and 50 mm/s (P=0.015). Moreover, the mean values in all intervals (P-R, Q-T and J-T) and T wave duration were higher in rabbits aged less than 12 mo. Likewise, R wave amplitude using filters was significantly higher in younger rabbits (0.108 vs. 0.097 mV; P=0.033) than in older rabbits.

### Comparison between male and female rabbits

Fourteen males (mean: 1.20±0.19 kg) were compared to 26 females (mean: 1.46±0.20 kg). Statistical results showed a significant difference in R wave amplitude using filters: R wave amplitude was greater in females (0.115 mV) than males (0.083 mV; P=0.012). Nevertheless, the R wave amplitude without filters did not show any differences. Moreover, body weight showed a significant difference between males and females (P<0.005).

### DISCUSSION

Firstly, our ECG measurements did not differ markedly from the previously reported reference values (Saitanov, 1960; Szabuniewicz et al., 1971; Meral et al., 1998; Fraser and Girling, 2009; Pariaut, 2009; Lord et al., 2010; Turner Giannico et al., 2015), Only P wave and R wave amplitudes were lower than some authors' reference values. These lower values could be due to the size of our rabbit breed and its smaller heart. As the P wave amplitude depends on the size of the atrium and the R wave amplitude depends on the mass of ventricular myocardium, a lower amplitude may be expected (Tilley, 1985; Edwards, 1987; Martin, 2001). Moreover, the S-T segment was isoelectric or presented a slight elevation, as had already been reported by Turner Giannico et al. (2015). On the other hand, there are no reference values for R wave amplitude without filters and J-T interval duration.

Regarding the QRS complex, the Rs pattern was the most frequent in lead II as Szabuniewicz et al. (1971) had reported before. However, this author had only described other different pattern (RS) in comparison with the other four patterns found in our study. In addition, we found spontaneous changes in QRS pattern in the subsequent recording of the same rabbit as Saitanov (1960), Szabuniewicz et al. (1971) and Lord et al. (2010) when body position was changed. As an example, Szabuniewicz et al. (1971) observed the following changes: rS↔R S↔Rs or Qr↔QR↔ qR. Szabuniewicz et al. (1971) proposed that these changes may be attributed to 3 causes: the looseness of the rabbit's skin, which produces shifting of electrodes, a vagal effect and the intrinsic electrochemical changes in the heart itself. Besides, Slapak and Hermanek (1957) suggested 'that variation in the position of the diaphragm due to ingestion of food caused variation in heart position and therefore in the QRS complex' (as quoted in Lord et al., 2010). However, this cause could be taken into less consideration than the rest, as the rabbits had no access to food between the different electrocardiograms in our research. The other 3 causes might have been produced in our study, especially the shifting of the electrodes, as they were again placed between the two electrocardiograms (sternal and dorsal recumbencies).

#### DIFFERENT ANALYSES OF PET RABBIT ELECTROCARDIOGRAM

Regarding body position, previous researchers in dogs (Hanton and Rabemampianina, 2006) or New Zealand rabbits (Saitanov, 1960) did not show differences according to this factor, although other researchers did (Rishniw et al., 2002; Harvey et al., 2005). In our research, body position should be taken into account when the J-T segment duration is assessed. As for other variables, there were no major differences, including heart rate or MEA. The fact that heart rate was similar in both recumbencies proves that the rabbits were not subjected to stress (Pariaut, 2009). So, the decision to use one or another recumbency method may be made depending on the facility of handling the rabbit.

The significant differences found between the 2 age groups are explained by the fact that each group had dissimilar heart rates. The faster heart rate in older rabbits caused a decrease in P-R and Q-T intervals, and in J-T segment duration (Tillev, 1985; Saitanov, 1960; Hanton and Rabemampianina, 2006; Bavegems et al., 2009), On the other hand, the R wave amplitude using filters showed significant differences between the 2 groups, although the values were in most of the reference ranges in both groups. This is caused because when power-line and anti-drift filters are ON, a voltage attenuation is produced. This is in line with the results obtained by Schrope et al. (1995) in cats.

Regarding statistical differences according to sex, the R wave amplitude using filters was the only difference. In humans, it has been observed that the S-T interval was shorter in men than women. This could be caused by sex hormones (Bidoggia et al., 2000). In contrast, there were no differences between both sexes in New Zealand rabbits (Turner Giannico et al., 2015) or in beagle dogs, so it is possible to carry out electrocardiographic analysis without taking this factor into account (Hanton and Rabemampianina, 2006). In our study, this variation in R wave amplitude may be caused by the difference in the body weight between males and females, as shown by Pearson's correlation, even though that correlation was not strong. This finding was also obtained by Lord et al. (2010), whose results showed a low correlation between R wave amplitude and body weight.

Our mean electrical axis results were similar to the reference values (Meral et al., 1998; Harkness and Turner, 2010; Lord et al., 2010) in the same species, but they were lower than those obtained by Szabuniewicz et al. (1971). Our values are narrower compared to those obtained by those authors. Moreover, MEA in pet rabbits was more leftward than other breeds, which can be attributed to the thoracic shape, which varies depending on breed (Lord et al., 2010). In our opinion, further research is needed to distinguish cardiac pathologies in rabbits.

Our findings show that it is important to evaluate MEA in an electrocardiogram with filters disabled because it will show a greater accuracy independently of the methods used to calculate the mean electrical axis, although MEA did not show significant differences between recumbencies, in contrast to results obtained in dogs (Rishniw et al., 2002) and cats (Harvey et al., 2005).

The major limitation to the present study may be the difficulty in separating young and old animals, as there were no rabbits aged over 20 mo. So, possible changes according to older healthy rabbits [>5 yr (Lennox, 2010)] cannot be analysed. Despite this, the probability of finding pathological rabbits could be higher than in younger animals (only 4 rabbits were pathological in our study, suspicion of RBBB).

#### CONCLUSIONS

On the basis of the results from this study, both recumbencies (sternal and dorsal) can be regarded as 2 useful positions for recording electrocardiograms to assess the heart's electrical activity in Netherland Dwarf rabbits with the exception of J-T duration, which is higher in dorsal recumbency. Moreover, R wave amplitude differences between both sexes and ages should be taken into account when electrocardiographic filters are enabled. Electrocardiographic filters also affect mean electrical axis; the strongest correlation was found between mean electrical axis calculated in electrocardiograms without electrocardiographic filters. However, filters are useful to avoid artefacts and to obtain a clear tracing ECG in order to measure waves and complex durations accurately. The mean electrical values are more leftward than in other species, so the clinician should be aware of this fact and the other specific electrocardiogram variables for Netherland Dwarf rabbit in order to ensure a good clinical examination.

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