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The canine T wave: a retrospective analysis on qualitative and quantitative T wave variables obtained in 129 healthy dogs and proposed reference intervals

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54 **Abbreviation table**

BW	body weight
FBs	French Bulldogs
MEA	mean electrical axis
RIs	reference intervals
R/T concordance	concordance between the R wave and T wave
Tpte	interval from the peak to the end of the T wave
Tpte/QT ratio	ratio between the duration of the Tpte and that of the QT interval
T/R %	ratio between the amplitude of the T wave and that of the R wave

55

56 **Introduction**

57           The surface electrocardiogram represents a non-invasive, inexpensive, and  
58 useful diagnostic tool which is widely available and has been routinely employed in  
59 small animal practice for decades [1]. It is primarily used to diagnose disturbances of  
60 cardiac rhythm; however, the electrocardiogram may also be used to monitor the  
61 response to antiarrhythmic drugs, unveil occult cardiomyopathies as well as cardiac  
62 chamber enlargement, and detect cardiac effects of extracardiac factors such as  
63 electrolyte abnormalities and drug toxicity [1,2]. The electrocardiographic waveforms  
64 are the expression of transmembrane action potentials of atrial and ventricular  
65 myocytes [1–3]. The main electrocardiographic components include the P wave,  
66 which represents atrial depolarization, the PQ interval, which represents

67 atrioventricular conduction time, the QRS complex, which represents ventricular  
68 depolarization, the ST segment and the T wave, which represent the ventricular  
69 repolarization, and the QT interval, which provides information on both ventricular  
70 depolarization and repolarization [1–3]. In human medicine, the study of  
71 repolarization represents a central research topic given its close association with  
72 ventricular diastolic function and arrhythmogenesis [4,5]. In contrast, historically,  
73 veterinary cardiologists have focused mainly on disturbances of depolarization rather  
74 than those of repolarization [6,7]. Moreover, many studies aimed at evaluating  
75 electrocardiographic variables in healthy dogs did not analyze important components  
76 of ventricular repolarization, such as the ST segment and T wave [8–12]. It should  
77 also be highlighted that the few available reports providing data on the T wave in  
78 healthy dogs are affected by relevant shortcomings, including small study  
79 populations [13–20], a restricted number of breeds enrolled [14,16–19], and/or limited  
80 T wave features investigated by each study [13–21]. Consequently, although the  
81 history of electrocardiography dates back to the 19th century, the T wave is still  
82 considered a poorly characterized and hardly interpretable electrocardiographic  
83 variable in dogs [2,3]. A robust background knowledge on the normal features of the  
84 canine T wave is essential for a proper interpretation of ventricular repolarization in  
85 the clinical setting, especially considering the role that repolarization alterations have  
86 in several canine cardiovascular conditions, such as myxomatous mitral valve  
87 disease, dilated cardiomyopathy, myocardial injury, familial QT prolongation, and  
88 heart failure [20,22–26]. Furthermore, such knowledge may be important for the early  
89 recognition of side effects of some frequently used drugs which have been  
90 demonstrated to be capable of altering ventricular repolarization and T wave  
91 features, both in humans and experimental canine models, such as sotalol [27,28].

92           Therefore, the purposes of this study were threefold: (1) to evaluate several  
93 qualitative and quantitative T wave variables in a large population of healthy dogs; (2)  
94 to assess the frequency distribution of selected T wave variables as well as their  
95 possible relationship with age, sex, body weight (BW) and somatotype; and (3) to  
96 provide reference intervals (RIs) for T wave quantitative variables.

97

## 98 **Animals, Materials, and Methods**

### 99 **Study population**

100           Medical records of healthy dogs that underwent an electrocardiogram as part  
101 of their diagnostic evaluation at the authors' institutions between January 2014 and  
102 June 2021 were retrospectively reviewed by a board-certified cardiologist (G.R.).  
103 Reasons for electrocardiographic analysis in an apparently healthy subject could  
104 include pre-operative evaluation before elective surgeries (e.g., castration, spaying)  
105 or screening for specific cardiac disorders in dogs from highly predisposed breeds.  
106 To be included, dogs had to be at least one year of age and have a complete case  
107 record, including signalment, history, clinical findings, a minimum laboratory  
108 database (including serum electrolytes) performed  $\leq$ six months from the examination,  
109 and cardiac investigation. The latter had to include at least a transthoracic  
110 echocardiography and one–two min six–lead electrocardiogram (leads I, II, III, aVR,  
111 aVL, and aVF) acquired at a paper speed of 50 mm/s and paper sensitivity of 10  
112 mm/mV, which had to be performed on the same day according to standardized  
113 techniques [1,29]. Dogs were considered healthy based on an unremarkable clinical  
114 history and physical examination as well as on normal laboratory,  
115 electrocardiographic, and echocardiographic findings. Dogs were excluded if they

116 had any cardiac or extra-cardiac disease as well as if they needed sedation or were  
117 receiving any therapy at the time of cardiovascular examination.

## 118 **Electrocardiographic recording and measurements**

119 In all dogs, an electrocardiogram was conducted with the dog positioned and  
120 manually restrained in right lateral recumbency, following the technique described by  
121 Tilley in 1992 [1]. All electrocardiograms were recorded in unsedated dogs using two  
122 commercially available machines<sup>e,f</sup>. For each animal, an effort was made to obtain an  
123 electrocardiographic tracing showing a clean baseline with easily recognizable  
124 waveforms. The same investigator (G.R.) manually measured intervals and  
125 amplitudes using a caliper and ruler with 0.5-mm graduations. Three representative  
126 consecutive beats were used to measure various electrocardiographic variables, and  
127 the results were averaged for each variable. Initially, the heart rhythm and  
128 conventional variables (i.e., heart rate; amplitude and duration of the P wave; PQ  
129 interval duration; R wave amplitude; duration and mean electrical axis in the frontal  
130 plane [MEA] of the QRS complex; and QT interval duration) were assessed  
131 according to the standard technique to evaluate if there were electrocardiographic  
132 abnormalities [2,3]. Such variables were judged to be normal/abnormal according to  
133 generic canine RIs [2]. Subsequently, according to the purposes of this study,  
134 particular attention was given to T wave qualitative and quantitative features.  
135 Qualitative variables were all assessed exclusively in lead II as previously described  
136 [2,14,17,20,26,30], and included:

137 (1) the T wave morphology (Fig. 1A–C): defined as “symmetrical” or  
138 “asymmetrical” if the T wave initial and terminal branches had a similar or  
139 clearly different slope, respectively. Moreover, it was noted whether the

140 asymmetricity was due to a gradual upstroke in the initial portion and a more  
141 rapid downstroke in the terminal portion, defined as “asymmetrical  
142 (slow/fast)”, or to an initial branch that was steeper than the terminal one,  
143 defined as “asymmetrical (fast/slow)”. Additional possible morphological  
144 patterns that were looked for included “biphasic” T waves (i.e., if T waves  
145 showed two peaks that moved in opposite directions in comparison to the  
146 baseline, one positive and one negative) and “bifid” (also known as “dome-  
147 and-dart”) T waves (i.e., if the ST segment and the first portion of the T wave  
148 formed a convex upward curve, followed by the terminal portion of the T  
149 wave, which formed a well-defined second positive peak separated from the  
150 first by a low amplitude negative deflection);

151 (2) the T wave polarity (Fig. 2A–C): T waves consisting of a single deflection  
152 could be classified as “positive” or “negative” according to the orientation of  
153 the wave’s peak in comparison to the baseline. In the case of biphasic T  
154 waves, three possible classifications were considered. Specifically, if the two  
155 peaks composing a biphasic T wave had different amplitudes, the T wave  
156 polarity was classified as positive when the positive peak’s amplitude was  
157 greater than the amplitude of the negative one, whereas it was classified as  
158 negative in the opposite case. Conversely, if the amplitudes of the two peaks  
159 moving in opposite directions were equal, the T wave polarity was classified  
160 as “neutral”;

161 (3) the concordance between the R and T waves (R/T concordance) (Fig. 2A–C):  
162 the term “concordant” was used when the R and T waves had the same  
163 polarity, while the term “discordant” was used in the opposite scenario. In the

164 case of a biphasic neutral T wave, the R/T concordance could not be  
165 evaluated.

166 Quantitative variables were assessed as previously described [2,14–16,18–  
167 21,26,27,31], and included:

168 (4) the T wave duration (Fig. 3A–C): measured from the beginning of the wave  
169 (i.e., the point where the initial branch of the T wave detached from the  
170 baseline) to its end (i.e., the intersection of a line tangential to the point of  
171 maximum slope of the terminal T wave with baseline level defined by the T-P  
172 segment) in lead II;

173 (5) the T wave amplitude (Fig. 3A–C): measured from the baseline to the peak of  
174 the wave in lead II. In the case of inconstant baseline associated with  
175 displacement of PQ interval relative to the TP one (e.g., PQ displacement due  
176 to atrial repolarization in patients with tall P waves), the latter interval was  
177 used as reference. In the case of a biphasic T wave, the amplitudes of both  
178 peaks were measured and noted separately;

179 (6) the ratio between the amplitude of the T wave and that of the R wave in lead  
180 II expressed in percentages (T/R %). Such a variable was not calculated in  
181 the case of biphasic T waves;

182 (7) the MEA of the T wave: calculated from leads I and aVF using the following  
183 equation:  $\text{MEA axis} = \arctan (I_{\text{amp}}, aVF_{\text{amp}}) \times 180/\pi$  (where lead  $I_{\text{amp}}$  is the  
184 amplitude of the T wave in lead I and  $aVF_{\text{amp}}$  is the amplitude of the T wave in  
185 lead aVF);

186 (8) the interval from the peak to the end of the T wave (Tpte) in lead II. In the  
187 case of a biphasic T wave, the interval between the nadir of the first  
188 component of the T wave and the end of the T wave was used (Fig. 4A–C);

189 (9) the ratio between the duration of the T<sub>pte</sub> and that of the QT interval (T<sub>pte</sub>/QT  
190 ratio).

## 191 **Statistical analysis**

192 All electrocardiographic data were collected into electronic spreadsheets<sup>g</sup> and  
193 then imported into a statistical software package<sup>h</sup> for further analysis. All continuous  
194 variables were tested for their distribution with a Shapiro–Wilk normality test. Initial  
195 descriptive statistics included mean ± standard deviation for normally distributed data  
196 and median and range (minimum to maximum) for data that were not normally  
197 distributed. For statistical analysis, when needed, dogs were divided according to  
198 their BW, age, and somatotype. Weight and age categories were adapted from  
199 previous literature [32,33], while somatotype categories were based on breed  
200 standards and conventional morphometric indices as previous done by other authors  
201 [12,34]. Specifically, dogs were purposefully classified as small (<15 kg) and  
202 medium-to-large dogs (≥15 kg), as adult (one–six years) and senior-to-geriatric dogs  
203 (≥seven years), and as brachymorphic and non-brachymorphic dogs (i.e.,  
204 mesomorphic and dolichomorphic dogs grouped together). Continuous and  
205 categorical variables were compared between male and female dogs as well as  
206 between dogs of distinct BW, age, and somatotype categories using the Mann–  
207 Whitney U test and  $\chi^2$  test, respectively. To further investigate the role of age and BW  
208 on continuous variables, a correlation analysis was also performed. This had the aim  
209 of excluding the possibility that the subdivision into categories (i.e., small/medium-to-  
210 large dogs and adult/senior-to-geriatric dogs) could obscure a statistically significant  
211 relationship of such patient variables on continuous T wave variables. For this  
212 purpose, Spearman’s correlation coefficients ( $r_s$ ) between T wave quantitative  
213 variables and age as well as BW were calculated. For correlation analysis, age and

214 BW were not categorized but were used as continuous variables. All variables were  
215 evaluated for identification of outliers by visual inspection of histograms and box plots  
216 and by Tukey's method [35,36]. Lastly, the 95% RIs of quantitative T wave variables  
217 were determined in the overall population using the nonparametric percentile method  
218 as recommended by the Clinical and Laboratory Standards Institute when the  
219 reference sample exceeds 120 subjects [35]. The 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles were  
220 defined as the lower and upper reference limits, respectively. As recommended, 90%  
221 confidence intervals around these limits were also calculated using nonparametric  
222 methods based on integer ranks, following Clinical and Laboratory Standards  
223 Institute guidelines [35]. In contrast, for smaller sample sizes (i.e., less than 120), RIs  
224 were determined using the Robust method and confidence intervals about their limits  
225 were bootstrapped as recommended<sup>h</sup> [35]. A value of  $P < 0.05$  was considered  
226 significant.

227

## 228 **Results**

229         Of the 132 that were initially recruited, three were excluded as the amount of  
230 artifact precluded an accurate analysis of the electrocardiographic tracings.  
231 Accordingly, the definitive study population was composed by 129 dogs. Forty-eight  
232 were male (42 entire and six castrated) and 81 were female (of which 72 entire and  
233 nine spayed) with a median age of three years (one–14 years). One hundred and  
234 eight and 21 were classified as adult and senior-to-geriatric dogs, respectively.  
235 Median BW was 12.2 kg (1.8–47 kg). Ninety-one and 38 were classified as small and  
236 medium-to-large dogs, respectively. Sixty-eight dogs were French Bulldogs (FBs); 14  
237 dogs were Doberman Pinschers; six dogs were Labrador Retrievers; five dogs each

238 were Golden Retrievers, Maltese, and Pug; four dogs were German Shepherd dogs;  
239 three dogs were Jack Russell Terriers; two dogs each were Boxers and Yorkshire  
240 Terriers; and the remaining were an Akita, Argentine Dogo, Australian Shepherd dog,  
241 Dachshund, Boston Terrier, Breton, Cane Corso, Cavalier King Charles Spaniel,  
242 Giant Schnauzer, Maremma Sheepdog, Miniature Pinscher, Miniature Schnauzer,  
243 Nova Scotia Duck Tolling Retriever, Rottweiler, and Vizsla. Seventy-three and 56  
244 were classified as brachymorphic and non-brachymorphic dogs, respectively. Mean  
245 heart rate was  $124.6 \pm 28.3$  beats per minute. One hundred and four had a regular  
246 sinus rhythm, while 25 dogs showed sinus arrhythmia. In the majority of dogs, all  
247 standard electrographic variables were within the generic canine RIs. Only few  
248 exceptions have been identified, all in FBs and all concerning the QRS complex  
249 MEA. Specifically, such an electrocardiographic variable was documented to be  
250 slightly below the generic lower reference limit ( $+40^\circ$  [2]) in 12 FBs (from  $+39^\circ$  to  
251  $+25^\circ$ ).

252       Regarding T wave qualitative variables, the T wave morphology, the T wave  
253 polarity, and the R/T concordance were considered not appropriately interpretable  
254 only in 2/129 (1.6%), in 1/129 (0.8%), and in 3/129 (2.3%) dogs, respectively.  
255 Moreover, an additional seven cases were not included in the statistical analysis on  
256 R/T concordance as this qualitative variable could not be calculated according to the  
257 methodology employed herein (i.e., dogs with biphasic neutral T waves).  
258 Consequently, statistical analysis on T wave morphology, T wave polarity, and R/T  
259 concordance was available for 127/129 (98.4%), 128/129 (99.2%), and 119/129  
260 (92.3%) dogs, respectively. In the overall population, T wave morphological patterns  
261 were distributed as follows: asymmetrical (slow/fast) in 67/127 (52.8%) dogs,  
262 symmetrical in 37/127 (29.1%) dogs, and biphasic in 23/127 (18.1%) dog; whereas

263 no dog showed a symmetrical (fast/slow) or bifid pattern. Moreover, the T wave  
264 polarity patterns were distributed as follows: positive in 98/128 (76.6%) dogs,  
265 negative in 23/128 (18%) dogs, and neutral in 7/128 (5.5%) dogs. Lastly, T and R  
266 waves were more commonly concordant (in 97/119 [81.5%] dogs) than discordant (in  
267 22/119 [18.5%] dogs). Interestingly, a similar frequency distribution was observed in  
268 each category (i.e., sex, age, BW and somatotype) for each qualitative variable (i.e.,  
269 T wave morphology, T wave polarity and R/T concordance). This finding was  
270 explained by the lack of statistically relevant differences when comparing T wave  
271 morphology, T wave polarity, and R/T concordance between male and female, small  
272 and medium-to-large dogs, adult and senior-to-geriatric dogs, and brachymorphic  
273 and non-brachymorphic dogs (Tables 1–3).

274 Similar findings were found for T wave quantitative variables, as no statistically  
275 relevant difference was found when comparing T wave duration, T wave amplitude, T  
276 wave MEA, T/R %, T<sub>p</sub>te, and T<sub>p</sub>te/QT ratio between male and female, small and  
277 medium-to-large dogs, adult and senior-to-geriatric dogs, and brachymorphic and  
278 non-brachymorphic dogs (Table 4).

279 Spearman's correlation coefficients are presented in Table 5. No statistically  
280 significant correlations between T wave quantitative variables and age or BW were  
281 found.

282 Potential outliers were identified neither for T wave duration and MEA nor for  
283 T/R %. Identified outliers for remaining variables (i.e., T wave amplitude, T<sub>p</sub>te and  
284 T<sub>p</sub>te/QT ratio) were individually investigated and retained for RIs calculation (Table  
285 6) because they were considered to be obtained from animals confirmed to be  
286 clinically healthy and not associated with errors in electrocardiographic

287 measurements [36,37]. Reference intervals were generated using the entire study  
288 population (i.e., 129 dogs) in the case of T wave duration, T wave MEA, T<sub>pte</sub>, and  
289 T<sub>pte</sub>/QT ratio. Concerning T/R %, its RI was generated from 106/129 (82.2%) dogs  
290 as this quantitative variable could not be calculated in 23 subjects (i.e., dogs with  
291 biphasic T waves) according to the methodology employed herein. Among dogs that  
292 allowed T/R % calculation, 75/106 (70.8%) subjects had a T/R % <25%, while 31/106  
293 (29.2%) subjects had a T/R % ≥25%. Even in the case of T wave amplitude, the RI  
294 was obtained from 106 dogs as the remaining 23 dogs had a biphasic morphological  
295 pattern. In these dogs, the amplitudes of two peaks composing their biphasic T  
296 waves were purposefully measured and noted separately. In dogs with biphasic T  
297 waves, the mean value of the positive peaks was +0.097 mV (± 0.043 mV); whereas,  
298 the median value of the negative peaks was -0.1 mV (from -0.5 to -0.05 mV).

299

## 300 **Discussion**

301 The T wave is one the most labile waves in the electrocardiogram and may  
302 change under several conditions, sometimes creating peculiar patterns that may  
303 suggest specific pathological states (e.g., deep/giant negative T waves secondary to  
304 myocardial injury [26], peaked T waves secondary to hyperkalemia [38]). Since an  
305 essential prerequisite to properly interpret T wave abnormalities is a thorough  
306 knowledge of its normal characteristics, we systematically analyzed several T wave  
307 features in a large population of healthy subjects, studying both qualitative and  
308 quantitative variables. Concerning qualitative variables, the main findings of the  
309 present study revealed that: (1) the asymmetrical (slow/fast) morphological pattern  
310 occurred most frequently, followed by the symmetrical and the biphasic ones,

311 whereas no healthy dog showed either a symmetrical (fast/slow) or bifid pattern; (2)  
312 positive T waves occurred most frequently, followed by negative and neutral ones;  
313 and (3) T and R waves were frequently, but not always, concordant in lead II.

314         On the electrocardiogram, the T wave represents the electrical inhomogeneity  
315 in the ventricular repolarization [2,3,39,40]. It is mainly caused by the electrical field  
316 generated by the temporal and spatial sequence of repolarization during phase 3 of  
317 the membrane action potential [2,3,39,40]. In contrast to depolarization, this phase  
318 has a lower voltage, since some repolarization has already occurred during phases 1  
319 and 2 (which correspond to the isoelectric ST segment on the electrocardiogram) and  
320 a much slower time course (as there is no specialized conduction system providing  
321 the pathway for repolarization) [3,39,40]. Furthermore, in normal conditions,  
322 repolarization is not a uniformly propagated phenomenon, but it occurs  
323 heterogeneously. Such heterogeneity, which may be found between the apex and  
324 the base and transmurally, is mainly due to differences in ion channel density  
325 (especially,  $I_{to}$ ,  $I_{Kr}$ , and  $I_{Ks}$ ) and action potential duration between epicardial,  
326 endocardial, and mid-myocardial cells (M cells) [3,39,40]. Given the above,  
327 ventricular repolarization normally proceeds from the epicardium to the endocardium  
328 as well as from the apex to the base, and repolarization velocity is slower in the  
329 subepicardial regions and faster in the subendocardial regions [2,3,36,37]. Such  
330 succession of electrical events determines the T wave configuration. In humans, the  
331 normal profile of the T wave is typically slightly asymmetric, with a slow ascending  
332 branch (the one associated with subepicardial repolarization) and faster descending  
333 branch (the one associated with subendocardial repolarization) [39,40]. Moreover,  
334 human T waves are normally concordant with the R waves as a consequence of the  
335 voltage gradient generated by opposing sequences of transmural depolarization and

336 repolarization (as depolarization, contrary to repolarization, progresses from  
337 endocardium to epicardium) [39,40].

338           However, it is important to acknowledge that the human T wave may be  
339 variable in shape under physiological conditions. For example, the T wave polarity  
340 may be negative until the age of 12–14 years (“juvenile T wave”) and then become  
341 positive after the age 16 years in many, but not all, adolescents [41]. Indeed,  
342 occasionally, negative T waves persist into adulthood, more frequently in women  
343 than in men [42,43]. Age and sex seem to influence even the human T wave  
344 morphology. For example, the incidence of bifid T waves may be relatively high in  
345 normal children [44]. In contrast, such a morphological pattern is infrequently  
346 encountered in adults, even more rarely if they are male [44,45]. Additional sources  
347 of variability are represented by ethnicity and levels of physical training [46]. Reasons  
348 for such variability are still a matter of debate in human medicine. Possible  
349 contributing factors may include differences in myocardial mass, myocardial tissue  
350 resistivity, extent of epicardial fat, endocrine status (especially concerning  
351 testosterone and estrogen levels), and chest conformation [46–48].

352           In light of the complexity of interpretation of human T waves, we felt it  
353 necessary not only to evaluate T wave qualitative variables in the entire study  
354 population, but also in selected categories. Interestingly, results from our overall  
355 population resembled those reported in the majority of healthy humans [39,40], as we  
356 frequently found asymmetrical (slow/fast) T waves that were positive and concordant  
357 with the R waves. Intriguingly, the higher prevalence of positive T waves, followed by  
358 that of negative and biphasic ones, were previously observed also by Vila et al. in 15  
359 healthy dogs [20]. However, contrary to human literature, we were unable to  
360 demonstrate any apparent difference related to age, sex, BW, or somatotype. In light

361 of the number of dogs enrolled herein, it seems unlikely that such a discrepancy may  
362 arise from a limited sample size, but it may rather be an expression of intrinsic  
363 species-related differences [49]. We also assume that some differences may become  
364 evident by investigating in future the effects on ventricular repolarization exerted by  
365 further possible influencing variables, such as the endocrine status (e.g., sex  
366 hormone levels), body condition score, respiration phase (e.g., inspiration versus  
367 expiration), and athletic level (e.g., working/sporting dogs versus companion dogs) of  
368 the enrolled subjects.

369         Concerning quantitative variables, we investigated several variables with the  
370 aim of providing statistically reliable RIs either for those commonly assessed in small  
371 animal practice (e.g., T wave duration and amplitude) and those that, although less  
372 familiar among veterinarians, have been reported to have clinically-relevant  
373 implications in dogs (e.g., T<sub>pte</sub> and T<sub>pte</sub>/QT ratio have been demonstrated to be  
374 good markers to predict ventricular arrhythmias and all-cause mortality in dogs with  
375 myxomatous mitral valve disease [20]). Although some previous studies reported  
376 mean and median values for some of these variables, it is important to highlight that  
377 the majority of them did not enroll enough dogs to be able to provide RIs with  
378 adequate statistical power [14–20]. However, some similarities between previously  
379 documented mean/median values and our median values can be noted, including  
380 those related to the T wave duration [17,19], the T<sub>pte</sub>, and the T<sub>pte</sub>/QT ratio [20]. A  
381 further similarity regards the median of amplitude values measured in dogs with  
382 positive and negative T waves [14,17,18,21]. Concerning the amplitudes of positive  
383 and negative peaks composing biphasic T waves, comparison with previous literature  
384 is limited by the fact that, although such a morphological pattern has been identified  
385 even by many investigators [13,14,20], only one study reported specific

386 measurements in this regard. Precisely, Hill [13], observed that the amplitude of both  
387 positive and negative peaks did not exceed 0.5 mV in dogs with biphasic T waves.  
388 Interestingly, this finding agrees with the results of our study. Concerning the T wave  
389 MEA, comparison is limited by the fact that, to our knowledge, only one previous  
390 study evaluated such a variable in healthy dogs [13]. Moreover, in that study, the  
391 author did not provide a RI representative of the entire study population, but  
392 presented results concerning T wave MEA distinguishing dogs with positive values  
393 from those with negative ones (i.e., they reported a MEA ranging from +26° to +180°  
394 in 35 dogs, and a MEA ranging from -45° to -147° in the remaining 35 dogs  
395 composing their study population) [13]. Nevertheless, it is intriguing to note that our  
396 minimum and maximum values (i.e., -146° and +180°) were almost identical to the  
397 lower negative and upper positive values previously documented [13]. A final  
398 consideration should be made concerning the T/R %. Although some veterinary  
399 textbooks state that the normal T wave should be <25% of the R wave amplitude in  
400 dogs [50–52], such a finding seems not to be supported by robust background  
401 evidence from original studies. Therefore, comparison between textbooks and our  
402 data should be made cautiously. Even if the majority (70.8%) of dogs enrolled herein  
403 had a T/R % <25% and the median T/R % value of our study population (i.e., 18%)  
404 fell into the cutoff mentioned by textbooks, it is interesting to note that some dogs  
405 (29.2%) had higher values and that the maximum value that we calculated (i.e.,  
406 71.4%) was remarkably beyond the aforesaid limit. Given the above, we believe that  
407 a T/R % <25% may represent an appropriate cutoff for many but not all dogs, and  
408 that a T/R % ≥25% should not be systematically interpreted as a certain pathological  
409 electrocardiographic finding, especially when this value is observed in healthy dogs  
410 with an otherwise normal electrocardiographic tracing. On the other hand, based on

411 our results, we can hypothesize that a T wave approximately  $\geq 75\%$  of the R wave  
412 amplitude should always raise concern and prompt further investigations (e.g.,  
413 assessment of serum potassium level to rule out hyperkalemia [38]).

414 As done for qualitative variables, we investigated the possible influence of  
415 age, sex, BW, and somatotype on quantitative variables. In line with the results from  
416 analysis on qualitative variables, we were unable to identify any statistically relevant  
417 influence of the aforesaid variables on T wave quantitative variables. Interestingly,  
418 this finding partially agreed with previous canine studies. Indeed, two studies found  
419 no effect of age and sex on T wave amplitude and duration [19,21], and another  
420 study demonstrated no statistically relevant differences in T wave amplitude among  
421 dogs of different sex and BW [17]. At the same time, it is important to underline that  
422 our results disagreed with human literature, as variables such as age, sex, and  
423 ethnicity have been demonstrated to influence T wave measurements in people  
424 [45,47,48,53]. As previously stated for qualitative variables, we do not know for  
425 certain whether this discrepancy may be due to intrinsic species-related differences  
426 or to the relatively limited number of variables investigated herein.

427 The results of the present study should be read in the context of certain  
428 limitations, first the retrospective design that prevented standardization of the time  
429 between electrocardiographic examinations and laboratory analysis. The fact that  
430 bloodwork was not systematically obtained on the day of electrocardiographic  
431 examination represents a limitation as it cannot be conclusively ruled out that some  
432 hematologic abnormality could have occurred at the time of the electrocardiogram.  
433 Nevertheless, it is unlikely that relevant laboratory changes may have arisen in the  
434 time between the blood tests and the electrocardiogram considering that all enrolled  
435 dogs appeared clinically healthy at the time of cardiological evaluation. The

436 retrospective design also prevented a systematic and standardized acclimation of  
437 dogs before electrocardiographic analysis. Second, our analysis was conducted  
438 exclusively on six-lead electrocardiograms, and almost all the T wave variables were  
439 assessed only in lead II as it represents the lead traditionally employed by  
440 veterinarians for electrocardiographic measurements [3,52]. Third, although the  
441 overall number of dogs enrolled was high, the number of subjects composing some  
442 categories was relatively low (e.g., the senior-to-geriatric dogs). Moreover, the  
443 sample size of groups composing each category was not always equally balanced  
444 (e.g., female and adult dogs were remarkably overrepresented compared to male  
445 and senior-to-geriatric dogs, respectively). Fourth, the high prevalence of FBs (likely  
446 caused by their recent exponential diffusion in our country [54, 55]) may have  
447 represented a source of bias; nevertheless, it was also useful, as it allowed us to  
448 create a wide group of brachymorphic dogs that almost proportionately  
449 counterbalanced the non-brachymorphic dogs. Concerning FBs, it should be also  
450 noticed that 12 dogs from this breed had a QRS complex MEA slightly below the  
451 generic RI provided by textbooks. Despite such a finding, we decided to maintain  
452 these dogs in our final analysis as they fulfilled all inclusion criteria (i.e., they were  
453 clinically healthy subjects with normal echocardiographic and laboratory findings) and  
454 their remaining electrocardiographic variables were within generic RIs. Our choice  
455 was also supported by data from a recent electrocardiographic survey in a large  
456 population of FBs demonstrating that a physiological left-shift of QRS complex MEA  
457 can be documented in healthy subjects from this breed, hypothetically due to their  
458 brachymorphic conformation rather than due to a true conduction disturbance [55].

## 459 **Conclusions**

460 In conclusion, a detailed description of several qualitative and quantitative  
461 features of the normal canine T waves is provided. Because our findings were  
462 obtained using a standardized electrocardiographic analysis in a large population of  
463 healthy dogs, they likely represent a reliable and broadly applicable guide for  
464 interpretation of the canine T wave.

465

#### 466 **Conflicts of interest**

467 None of the authors has a conflict of interest.

468

#### 469 **Footnotes**

470 <sup>e</sup> Cube ECG, Cardioline S.p.A., Caverano, Italy.

471 <sup>f</sup> ECG100S, Cardioline S.p.A., Italy.

472 <sup>g</sup> Microsoft Excel, version 2016, Microsoft Corporation, Redmond, Washington  
473 (USA).

474 <sup>h</sup> MedCalc Statistical Software version 19.5.1, Ostend, Belgium.

475

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633

## Figure legends

634

635 **Figure 1.** Selected close-ups of electrocardiographic tracings obtained in three  
636 healthy dogs. In all cases, a single complex recorded in lead II has been selected to  
637 illustrate in detail distinct T wave morphological patterns. A. Asymmetrical (slow/fast)  
638 T wave. B. Symmetrical T wave. C. Biphasic T wave.

639

640 **Figure 2.** Selected close-ups of electrocardiographic tracings obtained in three  
641 healthy dogs. In all cases, a single complex recorded in lead II has been selected to  
642 illustrate in detail distinct T wave polarity patterns as well as the concordance  
643 between the T wave and the R wave. A. Positive T wave that is concordant with the  
644 R wave. B. Negative T wave that is discordant with the R wave. C. Neutral T wave.  
645 Note that the T wave morphology is biphasic and the two opposite peaks have an  
646 almost identical amplitude. In this case, concordance between the T wave and the R  
647 wave cannot be established.

648

649 **Figure 3.** Selected close-ups of electrocardiographic tracings obtained in three  
650 healthy dogs. In all cases, a single complex recorded in lead II has been selected to  
651 illustrate in detail how T wave amplitude and duration have been measured. A.  
652 Measurements in a dog with a positive T wave. B. Measurements in a dog with a  
653 negative T wave. C. Measurements in a dog with a biphasic T wave. In each panel,  
654 the blue dotted lines help to identify the baseline, the red dotted lines the start and  
655 the end of the T waves, and the green dotted lines the T wave amplitudes.

656

657 **Figure 4.** Selected close-ups of electrocardiographic tracings obtained in the same  
658 three healthy dogs from Figure 3. In all cases, a single complex recorded in lead II  
659 has been selected to illustrate in detail how Tpte has been measured according to  
660 previous indications [20]. A. Measurement in a dog with a positive T wave. B.  
661 Measurement in a dog with a negative T wave. C. Measurement in a dog with a  
662 biphasic T wave. In each panel, the blue dotted lines help to identify the baseline,  
663 while the red dotted lines the reference points for the measurement of Tpte. Tpte: T  
664 wave peak-end interval duration.