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# Use of a spot-check protocol to measure ventricular response rate in dogs with atrial fibrillation☆

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**KEYWORDS**
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**Abstract** *Introduction/Objectives:* Fast ventricular response rates (VRRs) have negative prognostic value in atrial fibrillation (AF). Therapeutic recommendations for canine AF rely on measurements of VRR from ambulatory electrocardiograms (aECGs).
*Animals, Materials and Methods:* Data from aECG of dogs with AF were prospectively acquired alongside VRR measurements obtained at home using a smartphone-based ECG device between 7:00 and 23:00. From these data, three protocols were outlined: (A) three eight-hourly spot-checks, (B) three six-hourly spot-checks, and (C) five predetermined spot-checks. The performance of the protocols was compared with aECG using mesor, mean and median VRR. The presence of circadian variation was explored using cosinor analysis.
*Results:* Eighteen aECGs were analysed, and 14 were used to test the spot-check protocols. The protocols showed moderate to strong correlation with aECG, with protocol B performing best (mean, $r = 0.84$, $P=<0.0001$, bias $-22.96$, limits of agreement 24.4; median, $r = 0.90$, $P<0.0001$, bias $-24.48$, limits of agreement 22.04). Using a mean VRR $<125$ beats per minute (bpm) as cut-off, 10 of 14 dogs (71.4%) had inadequate VRR control. All protocols correctly identified these dogs, with protocol A performing best for mean VRR $>140$ bpm. Circadian variation in VRR was identified in 17 of 18 dogs (94%).

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*Limitations:* This study is limited by the small population, highly prevalent inadequate VRR, non-standardised treatment, measurement artefacts and precise temporal alignment with aECG.
*Conclusions:* Spot-check protocols offer a practical, affordable alternative to aECG for VRR assessment in dogs with AF. A mean VRR >140 bpm in the protocol predicts inadequate rate control on aECG.


**Abbreviations**

| | |
|---|---|
| AF | atrial fibrillation |
| aECG | ambulatory electrocardiogram |
| Bpm | beats per minute |
| CV | circadian variation |
| GSD | German Shepherd Dog |
| HR | heart rate |
| LOA | limits of agreement |
| VRR | ventricular response rate |

## Introduction

The management of canine atrial fibrillation (AF) is an important focus of clinical research as it is frequently encountered in small-animal practice [1] as a complication of commonly acquired [2—4] and congenital cardiac diseases or as lone AF (i.e. in the absence of evident structural heart disease) [1]. Patients with chronic AF and poor control of the ventricular response rate (VRR) have a poorer prognosis [5—7], possibly due to the inherent systolic dysfunction associated with a fast and irregular VRR [8].

In dogs with AF, differences between in-clinic electrocardiograms (ECGs) and ambulatory ECGs (aECGs) have highlighted the importance of obtaining a comprehensive, representative overview of the dogs' VRR in the home setting rather than at one moment in time in the clinic, when greater sympathetic stimulation is likely to bias measurements [9]. Based on longitudinal studies that include aECG data, cut-offs for target 24-h mean VRR of <140 beats per minute (bpm) [9,10] and, more recently, <125 bpm currently guide treatment towards improving outcomes by reducing VRR with negative chronotropes [5—7].

Circadian (sinusoidal) variation (CV) in VRR has been previously identified in dogs with sinus rhythm and in most dogs with atrial fibrillation [11]. This was the first time that CV was studied in dogs from a clinical population by cosinor analysis, although it had been demonstrated in previous studies conducted in experimental settings [12,13].

Cosinor analysis is a method used to study daily rhythms by fitting a sinusoidal wave to the data. This method helps estimate a rhythm-adjusted mean called the mesor (midline statistic of rhythm), how much the values rise and fall (amplitude) and when the highest point occurs (acrophase). In circadian studies, the period lasts 24 h and the curve can be fitted using regression analysis based on the time each measurement was taken [15].

Furthermore, where circadian variation exists, the VRR's mesor is better suited as a measure of central tendency (i.e. the headline 24-h value of VRR) than the mean VRR [14,15]. The mesor has been used to validate retrospectively the use of mean and median VRRs as surrogates for daily rate control in canine AF. Their use still needs to be validated prospectively against the mesor from aECG.

Ambulatory ECG recordings have an associated cost and are not widely available outside of referral centres. They can be disruptive to the owners' and pets' routines and may not be tolerated by some patients with AF [9]. They are compounded by the frequent need for consecutive aECGs in individual patients until a desired mean VRR is reached [3,6]. Therefore, there is an unmet need for alternatives to out-of-clinic measurement of VRR [16] that provide representative daily VRRs by capturing the influence of different times of day and activity levels. The ability of spot-check protocols to estimate central measures of VRR in dogs with AF has been studied retrospectively, demonstrating a good correlation with the measures obtained with aECG [11].

The accuracy of smartphone-based ECG devices to obtain readings of heart rate in the home environment has been studied in canine patients [16,17], with an overall good level of acceptance from dog owners [18]. They provide mean heart rates or VRRs from short recording periods of 30 s. since the use of mean VRRs against the mesor has been validated, these devices offer potential for determining VRR control, but their usefulness in

the management of dogs with AF has remained unknown until now.

The hypothesis of the study is that the measures of VRR obtained from the spot-check protocols would be equivalent to those obtained from aECG and would identify patients with adequate control of AF.

To investigate this hypothesis, three spot-check protocols for ECG recording were designed, for owners to follow at home using a validated smartphone-based ECG device.[a]

The primary goal of this study was to test these protocols prospectively and compare the VRR from each protocol with a simultaneously derived aECG mesor.

## Animals, materials and methods

This was a prospective single-centre study approved by the University of Edinburgh Veterinary Ethical Research Committee (reference number 148.19).

Owners of dogs presented to the Cardiology Service between July 2021 and December 2022 that required an aECG to manage their AF were invited to enrol their pet in the study. Signed informed consent was obtained. Demographic data, including the presence of congestive heart failure, were collected. More than one aECG was allowed from an individual patient if it fulfilled the inclusion criteria.

Dogs' hair was clipped as per hospital protocol for a three-channel aECG monitor.[b] One larger area on each hemithorax allowed the attachment of five electrode pads on the thorax, with two additional small clip patches caudal to the initial ones to allow application of a smartphone-based ECG device[a] onto the skin (figure available in Supplementary Figure 1, available online). The device was tested to ensure optimal location prior to patient discharge.

Owners were loaned a smartphone-based ECG device[a] and asked to download the corresponding phone application onto their personal mobile phone.[c] On discharge from the hospital, owners were taught how to record an ECG with the device for a duration of 30 s at prescribed times of the day (07:00, 13:00, 15:00, 19:00, 23:00), allowing a period of acclimatisation after placing the device against the skin. Lubricating jelly[d] was provided to optimise the quality of the recordings.

During the aECG recording period, owners were asked to replicate their normal daily routine and record their pet's activities in a diary. Owners had access to a prerecorded instruction video for further assistance. Ambulatory ECGs were not standardised to start at the same time.

Once the recording period was completed, owners removed the aECG monitor at home. They posted the devices and the activity diary to the investigators, emailing the app-generated PDF of the ECGs to the cardiology service.

Data from aECG recordings were stored on a 128-MB removable CF card and transferred to a hard drive for analysis with commercial software.[e] All recordings were analysed automatically and manually corrected after their acquisition by a board-certified cardiologist or a cardiology resident under supervision. Analyses included manual confirmation that R-R intervals had been recognised by the software and that QRS complexes had been correctly labelled as supraventricular or ventricular, with relabelling of complexes as required. Artefact was defined as disruption to the tracing in all three leads to a degree that did not allow recognition of the QRS complexes.

Exclusion criteria based on aECG included the following: intermittent AF, concomitant arrhythmias such as atrial flutter or complex ventricular arrhythmias grade $\geq$2 (as previously described [14]), recordings of $<$23 h duration and the presence of more than 10% artefact. Dogs were also excluded based on smartphone-based ECG recordings when there was a lack of owner compliance such as insufficient recordings, recordings outside of the specified times by $>$1 h or sufficient artefact to impede VRR calculation. If an aECG recording lasted more than 24 h, the studied period included only the 24 h that had simultaneous smartphone-based ECG recordings.

### Data analysis

Ventricular response rate measurements obtained from the smartphone-based ECG device (average VRR calculated automatically by the smartphone application) were inserted into a spreadsheet, and the mean and median VRRs were calculated by three different spot-check protocols (A, B and C). The mean, median and mesor (when CV was present) of the aECG VRR were calculated by the previously described protocol [11]. Briefly, once

[a] AliveCor Veterinary Heart Monitor, AliveCor®, Mountain View, California, USA.
[b] Vista Access or Vista Plus, Novacor UK, Swanley, Kent, UK.
[c] KardiaMobile, AliveCor®, Mountain View, California, USA.
[d] Optilube™ Sterile Lubricating Jelly, Optimum Medical, Leeds, West Yorkshire, UK.
[e] Novacor HolterSoft Ultima Version 2.5.5, Novacor UK, Swanley, Kent, UK.

the aECG data had been analysed, R-R intervals (milliseconds) were exported into a spreadsheet and transformed into instantaneous heart rate (HR) using the formula HR = 60,000/R-R. These values were then averaged into each one of the 24 h (24 data points per dog), for mean, median and mesor calculation.

For purposes of clarity, 'average VRR' is used to describe the arithmetic mean of VRRs calculated directly from R-R intervals and that were used to generate a mesor by cosinor analysis [11,19,20]. The terms 'mean VRR' and 'median VRR' are restricted to the arithmetic mean and median of average VRRs that were compared with the mesor, as outlined below.

The following representations of VRR were obtained:

1) Ambulatory ECG VRR (taken from entire ambulatory 24-h ECG recording);
   a. The mean, median and mesor of the 24-hourly average VRRs;
2) Spot-check VRR (taken from home ECG readings);

**Protocol A:** the mean and median of three spot-check measurements taken eight hours apart (7:00, 15:00, 23:00);

**Protocol B:** The mean and median of three spot-check measurements taken six hours apart during daytime (7:00, 13:00, 19:00);

**Protocol C:** The mean and median of five spot-check measurements (7:00, 13:00, 15:00, 19:00, 23:00);

## Statistical analysis

Statistical analysis was performed using commercially available software.[f,g,h] Normality testing was performed with visual inspection of data and the Anderson-Darling test. All VRR data are presented with the range of values. All other data are presented as mean and 95% confidence intervals or median and range depending on normality of distribution.

Once CV had been confirmed by cosinor analysis, the correlations of the mean and median VRR (aECG and spot-check protocols) with the mesor VRR (aECG only) were determined by Pearson's correlation (normal distribution) or Spearman's rho tests (not normally distributed) as appropriate. The mean and median VRR obtained with spot-check protocols were also tested for correlation with the mean and median of aECG data using the same tests. Strength of correlation was based on r values as follows: negligible (0.00—0.10), weak (0.10—0.39), moderate (0.40—0.69), strong (0.70—0.89) or very strong (0.90—1.0) [21]. Where correlations were moderate to very strong, bias and limits of agreement (LOA) were determined with Bland-Altman plots.

The ability of the spot-check protocols to detect patients with inadequate control of AF was tested by calculating the accuracy, sensitivity, specificity, positive and negative predictive values [22] as outlined (Supplementary Figure II, available online). Patients with inadequate control of AF were defined by two previously reported criteria:

a) Mean aECG VRR >125 bpm [5,6]
b) Mean aECG VRR >140 bpm [9,10]

Statistical significance for all tests was set at $P<0.05$.

## Results

Twenty-one aECG recordings and spot-check HR data were obtained from 18 dogs diagnosed with AF. Of these, three sets of data were excluded due to poor-quality aECG and four due to poor owner compliance with the spot-check protocols. A total of 18 aECG recordings were used for descriptive and cosinor analysis, but only 14 were used to test the spot-check protocols (Fig. 1).

Demographic data of the population were calculated (Table 1). There were more male dogs (15/18, 83.3%) than female dogs (3/18, 16.7%). The average age was 7.1 years (5.5—10.0 years) with an average weight of 42.08 kg (20.2—51.4 kg). Fifteen dogs had stable congestive heart failure at the time of the ambulatory ECG recording (15/18, 83.3%).

## Agreement between measures of central tendency in the ambulatory electrocardiogram recording

From the 18 aECGs, the average median VRR was 148.3 bpm (65.6—186.5 bpm) and the average mean was 148.4 bpm (71.8—183.6 bpm). Circadian variation in VRR was identified in 17 of 18 recordings (94%), with an average mesor of 146.7 bpm (71.9—189.7 bpm) and an average amplitude of 15 bpm (4.3—35.7 bpm). The mean and median VRR

[f] Microsoft Excel 2020 version 16.52 (21080801), Microsoft Corporation, Redmond, Washington, USA.
[g] Minitab 17.1.0, Minitab LLC, State College, Pennsylvania, USA.
[h] GraphPad Prism 8, GraphPad Software, San Diego, California, USA.

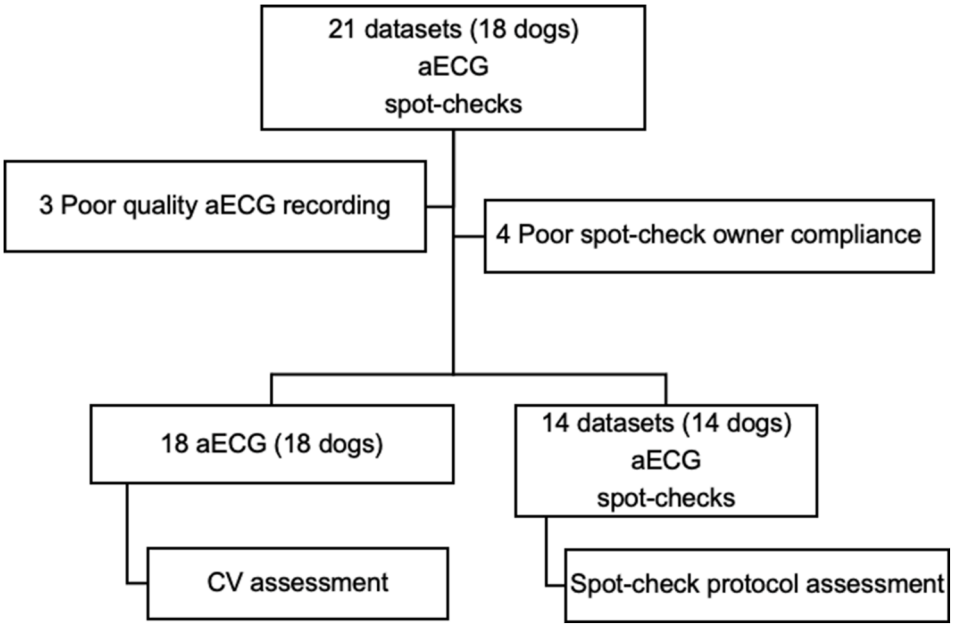


**Figure 1** Diagram demonstrating the study group of 18 dogs with atrial fibrillation, which generated 21 datasets of ambulatory electrocardiogram data and home-recordings of the heart rate. Seven datasets were excluded, resulting in 14 datasets that were used to test the three spot-check protocols. aECG: ambulatory electrocardiogram; CV: circadian variation; VRR: ventricular response rate.

both correlated very strongly with the mesor and with each other. The mean had a marginally smaller negative bias from the mesor than the median did, and the LOA were narrower (Fig. 2).

## Agreement between the spot-check protocols and ambulatory electrocardiogram data

In the 14 dogs with both aECG and spot-check protocol data, circadian variation was present, and so mean and median VRR from each spot-check protocol were compared against the mesor (Fig. 3, Table 2), as well as the mean and median values obtained from the aECG (Table 2).

Similar correlations and biases were obtained between each protocol and the mesor from aECG data. Protocol B had the strongest correlations for the median and the narrowest LOA (Table 2), although protocol A also generated strong to very strong correlations. Although the bias was lower for protocol C with acceptable LOA, the correlation with the mesor was only moderate.

The VRR was obtained from each spot-check protocol and aECG using the calculations for mean, median and mesor (aECG data only) in the 14 dogs from which both sets of data were obtained (Table 3). Using the previously published 125 bpm cut-off for mean VRR from aECG, four of 14 dogs (28.6%) were identified as having adequate control of their VRR. The mesor correctly identified these four dogs. Of these, three of four were correctly recognised by at least two spot-check protocols and one of four was identified only by the median, but not the mean of protocol A. All 10 of 14 dogs (71.4%) with inadequate control of the VRR were correctly identified by each spot-check protocol.

The accuracy, sensitivity, specificity, and positive and negative predictive values were calculated for the mean VRR obtained from each spot-check protocol to identify inadequate control of the VRR obtained from the aECG (Table 4). All protocols performed better at identifying patients with a mean VRR $>$140 bpm than 125 bpm. Protocol A showed the best performance with 100 % across sensitivity, specificity, positive and negative predictive value and accuracy. Protocol C performed the worst at identifying this cut-off.

## Discussion

This study demonstrates that home ECG recordings obtained using a smartphone-based ECG device can accurately measure central tendencies of VRR in dogs with AF using a spot-check protocol. It builds on a previous retrospective study that showed that spot-checks at 4-, 6- and 8-h intervals would have adequately estimated the median VRR obtained from 24-h aECGs [11]. It has now been

**Table 1** Descriptive data of the study group of 18 dogs, including diagnosis, treatment and presence of circadian variation (sinusoidal distribution) on 24-h ambulatory ECG.

| Breed | Age (years) | Gender and neutered status | Weight (kg) | Treatment | CHF | Cardiac disease | Circadian variation |
|---|---|---|---|---|---|---|---|
| Labrador retriever | 10 | MN | 42.7 | Furosemide, pimobendan, diltiazem, digoxin | Yes | MMVD | Yes |
| GSD | 8 | MN | 39.8 | Furosemide, pimobendan, diltiazem, potassium chloride | Yes | MMVD | No |
| Akita | 11 | FE | 49.5 | Furosemide, pimobendan, benazepril/spironolactone | Yes | Bilateral atrial enlargement — open diagnosis | Yes |
| Great Dane | 7 | ME | 83 | Pimobendan, diltiazem | No | DCM | Yes |
| Labrador retriever | 10 | ME | 43.5 | Furosemide, pimobendan, benazepril/spironolactone, digoxin, potassium chloride, maropitant | Yes | MMVD | Yes |
| Greyhound | 11 | MN | 31.7 | Furosemide, pimobendan, benazepril/spironolactone and sotalol | Yes | MMVD + ventricular arrhythmia | Yes |
| Great Dane | 2 | ME | 58.6 | Furosemide, pimobendan, diltiazem | Yes | DCM + AVD | Yes |
| Doberman pinscher | 7 | MN | 57.25 | Furosemide, pimobendan, benazepril/spironolactone, diltiazem and levothyroxine | Yes | DCM | Yes |
| Boxer dog | 9 | ME | 39.1 | Furosemide, pimobendan, trilostane | Yes | ARVC, heart base mass, ventricular arrhythmia | Yes |
| Labradoodle | 6 | FN | 20.2 | Furosemide, pimobendan, benazepril/spironolactone | Yes | MVD | Not tested |
| Hungarian Vizla | 11 | MN | 32.8 | None | No | Lone AF | Not tested |
| GSD | 9 | ME | 20.5 | Torasemide, pimobendan, benazepril/spironolactone, amiloride/hydrochlorothiazide, diltiazem | Yes | MMVD | Yes |
| Crossbreed | 1 | ME | 29.9 | Furosemide, pimobendan, digoxin | Yes | DCM | Yes |
| Rottweiler | 2 | FE | 34.5 | Furosemide | Yes | MVD, SAS | Yes |
| Dogue de Bordeaux | 7 | ME | 49.5 | Pimobendan | No | AV valve regurgitation; Systolic dysfunction | Yes |
| Labrador retriever | 4 | MN | 25.8 | Torasemide, pimobendan, benazepril/spironolactone, diltiazem, digoxin | Yes | MVD, post MV repair with mitral valve stenosis | Yes |
| Dogue de Bordeaux | 6 | ME | 66.55 | Furosemide, pimobendan | Yes | TICM | Yes |
| Weimaraner | 7 | ME | 32.5 | Furosemide, pimobendan, benazepril | Yes | MMVD | Yes |

aECG: ambulatory electrocardiogram; AF: atrial fibrillation; AV: atrioventricular; CHF: congestive heart failure; DCM: dilated cardiomyopathy; FE: female entire; FN: female neutered; GSD, German Shepherd Dog; ME: male entire; MMVD: myxomatous mitral valve disease; MN: male neutered; MV: mitral valve; MVD: mitral valve dysplasia; PDA: patent ductus arteriosus; SAS: subaortic stenosis; TICM: tachycardia induced cardiomyopathy.

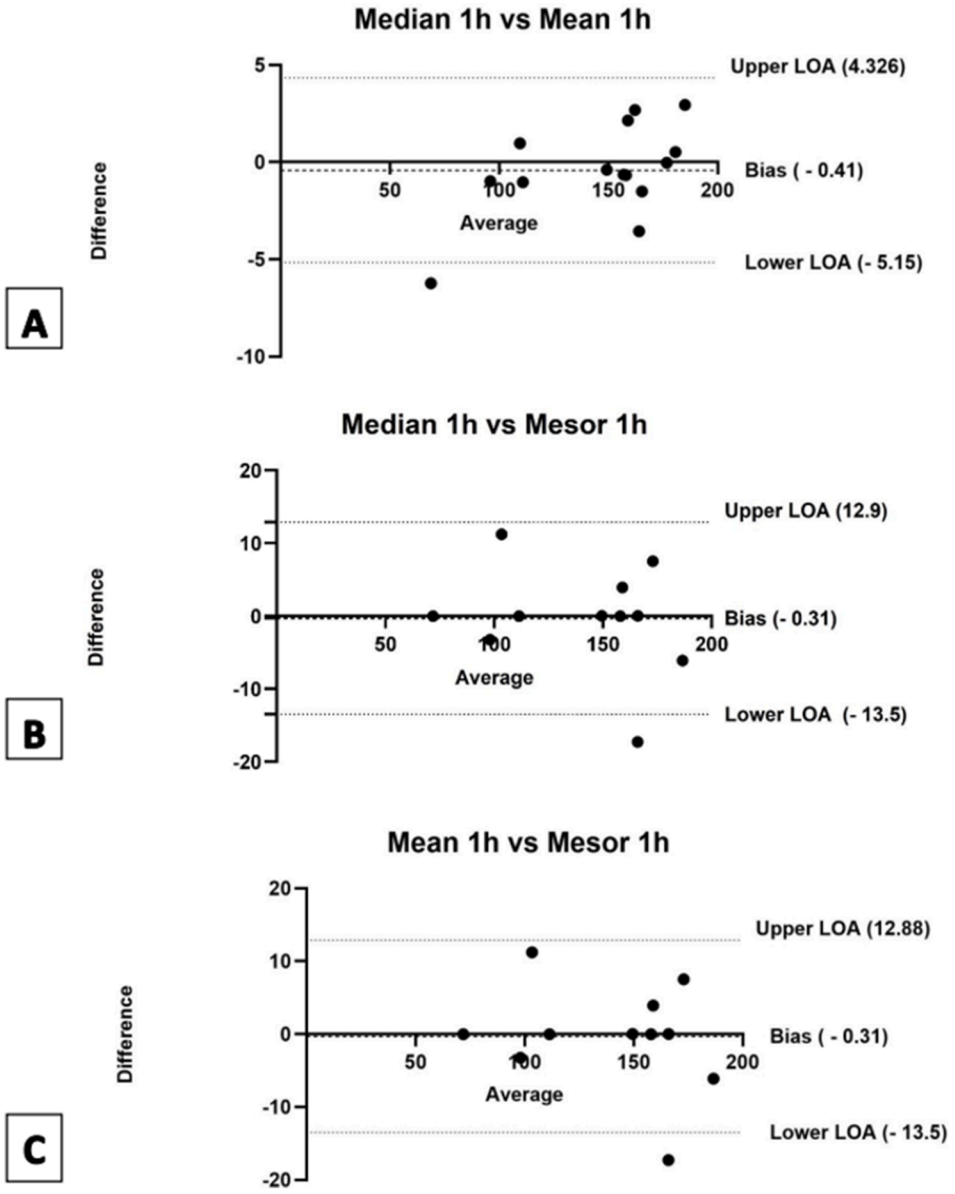


**Figure 2** Bland-Altman plots comparing median and mean ventricular response rates from ambulatory electrocardiogram data between each other (panel A) and against the mesor (Mesor 1 h, panels B and C, respectively) in 17 dogs that had circadian (sinusoidal) variation in their heart rates on ambulatory electrocardiogram recordings. The median, mean and mesor of the 24-hourly average VRRs are represented as Median 1 h, Mean 1 h and Mesor 1 h. A very strong correlation was found between mean and mesor ($r = 0.98$, $P=<0.0001$, bias $= -0.31$, LOA $= 6.73$) and median and mesor ($r = 0.98$, $P=<0.0001$, bias $= -0.79$, LOA $= 7.36$). The correlation between mean and median was also very strong ($r = 0.99$, $P=<0.0001$, bias $= -0.41$, LOA $= 2.42$). aECG: ambulatory electrocardiogram; LOA: limits of agreement.

shown, prospectively, that an owner could monitor their dog's VRR by following one of the suggested protocols with the potential to reach better rate control in pet dogs with AF for which aECG is not feasible.

Surprisingly, when tested prospectively, the protocol with more measurements (protocol C with five measures as opposed to three in protocols A and B) showed the weakest correlation with aECG. Protocol A had a strong correlation with aECG but had a higher risk of overestimating the real measurement than the other protocols. Protocol B had a smaller risk of overestimation and the strongest correlation with the mesor from aECG data. In the retrospective study, the protocol of sampling at six-hourly intervals had four measurements instead of the three measurements used in protocol B, whereas the protocol sampling at

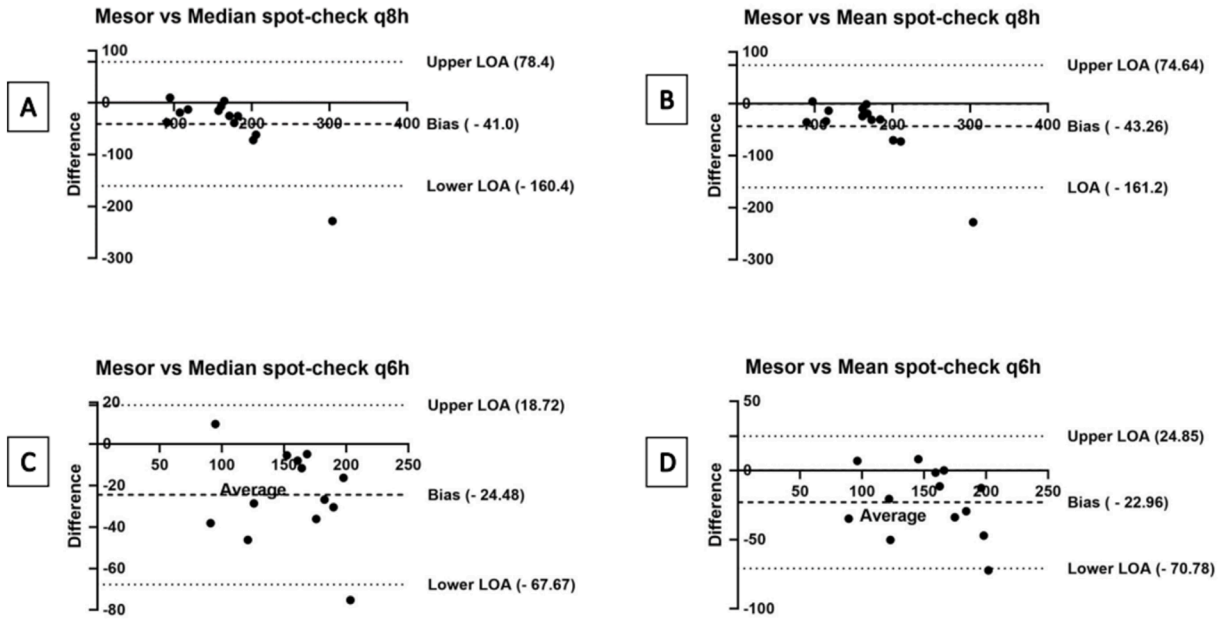


**Figure 3** Four Bland-Altman plots generated from spot-check smartphone-based electrocardiogram recordings taken every eight (protocol A, panels A and B) and six (protocol B, panels C and D) hours. Median and mean ventricular response rates (VRRs) spot-check measurements were compared with mesor VRR values in a population of dogs with atrial fibrillation. LOA: limits of agreement; q 6 h: every six hours; q 8 h: every eight hours.

eight-h intervals had three measurements, as used for protocol A. Given that protocol B outperformed protocol A, there may be an additional advantage to obtaining four measurements every six h. However, this was not investigated in this study protocols were created to avoid interference with the owners' sleeping schedules to improve compliance. Furthermore, it appears that measuring more frequently is not better than measuring less frequently. As VRR varies in a circadian manner in AF dogs, it dips at night. Therefore, too many spot-checks during the day likely over-represent the higher daytime values.

In terms of ability to identify cases with inadequate mean VRR control [5—7,9], protocol A outperformed the others, especially when using the median VRR of the spot-checks. The type of analysis that was used to assess this is highly reliant on population numbers because one case represents a high percentage of the population for a false-negative or false-positive result. For this reason, caution is recommended when interpreting them, the authors recommend caution when interpreting them.

In the current study, additional human and technical factors such as the ability of owners to take VRR measurements of their pets at home using a smartphone-based ECG device[b] were taken into consideration. Clipping the fur and providing lubricating gel helped to improve the quality of the smartphone-based ECG recording. However, even with a detailed information sheet showing time points at which to obtain measurements, some owners did not comply with the instructions, and one of them did not achieve measurements due to the poor quality of the recording.

The importance of achieving adequate rate control in canine patients with AF equates to longer survival and has been proposed as mean VRR $<$125 bpm and $<$140 bpm in aECG and HR $<$155 bpm in in-clinic ECG [5—7,9]. Importantly, the benefit is apparent even when adequate control of VRR is only achieved after several therapeutic modifications over time, guided by serial evaluation with aECG [7]. For these reasons, it is important to offer serial monitoring that is accurate but that removes the expense of serial aECG.

Up until now, the only proven alternative has been the use of single in-clinic ECG. However, this provides only limited insights into the daily VRR because it can be highly impacted by sympathetic tone and excitement within the clinic [9]. Whether the spot-check protocols compare favourably with aECG has been determined, but also if they outperform single in-clinic ECG not only whether spot-check protocols compare favourably with aECG but also if they outperform single in-clinic ECG. It has been shown that in-clinic ECG rates $<$160 bpm could identify patients with mean VRR $\leq$140 bpm in aECG with 91% sensitivity and specificity, whereas in-clinic ECG rates $\leq$155 bpm showed 73% sensitivity with 100% specificity [9]. In this study, the mean VRR

**Table 2** Results of correlation and Bland-Altman analyses comparing median and mean ventricular response rates (VRRs) obtained from three spot-check protocols against the mesor calculated from ambulatory ECG data in 14 dogs with atrial fibrillation. Mean and median VRRs from each protocol are also compared with mean and median ambulatory ECG data. Protocol A: three spot-checks measured eight h apart; protocol B: three spot-checks measured six h apart during daytime; protocol C: five spot-checks.

| Protocol | Spot-check vs aECG | Correlation (r) | P | Bias | LOA |
|---|---|---|---|---|---|
| A | Mean vs mesor | Strong (0.86) | <0.0001 | −43.26 | 60.15 |
| | Median vs mesor | Strong (0.81) | 0.001 | −41.3 | 60.92 |
| | Mean vs mean | Strong (0.7) | 0.0001 | −41.83 | 60.86 |
| | Median vs median | Moderate (r = 0.66) | 0.01 | −41.74 | 60.6 |
| B | Mean vs mesor | Strong (0.84) | <0.0001 | −22.96 | 24.4 |
| | Median vs mesor | Very strong (0.90) | <0.0001 | −24.48 | 22.04 |
| | Mean vs mean | Strong (r = 0.76) | 0.03 | −23.1 | 24.16 |
| | Median vs median | Strong (0.79) | 0.002 | −26.11 | 22.41 |
| C | Mean vs mesor | Moderate (0.62) | 0.02 | −14.86 | 31.59 |
| | Median vs mesor | Moderate (0.61) | 0.03 | −16.74 | 32.38 |
| | Mean vs mean | Moderate (0.64) | 0.014 | −16.77 | 31.08 |
| | Median vs median | Moderate (0.59) | 0.026 | −17.36 | 33.16 |

aECG: ambulatory electrocardiogram; LOA: limits of agreement.

calculated from protocols A and B had higher sensitivity and specificity for identifying patients with VRR >140 bpm on aECG in a population of dogs that was heavily biased towards dogs with inadequate rate control. In other words, in a clinical case where spot-check protocols detect a VRR >140 bpm, there is an extremely high likelihood that the dog does not have good rate control and may benefit from escalation of antiarrhythmic medication.

The spot-check protocols were designed from a initial retrospective study that used the mesor as the gold-standard headline figure of 24-hourly daily heart rate [11,15]. In that study and in the current study, the mesor could be accurately represented by both the mean and median values for the 24-h period. This is important because cosinor analysis is complex, is not easily calculated in clinical environments and is not routinely provided by aECG software analysis [20]. Furthermore, it validates the use of mean 24-hourly values from aECG as target cut-offs for 24-hourly VRR, and the use of spot-check protocols, which, in themselves, generate mean values. Although this study did not compare the spot-check protocols with in-clinic ECG, the results suggest that the spot-check protocol is a promising and affordable alternative to aECG and probably superior to in-clinic ECG. Obtaining the median value from three home measurements is remarkably simple, making this approach an especially attractive option compared to other available methods. However, it is important to ensure that each recording is of high quality and accurately reflects the true rhythm. In this study, investigators requested the PDF output from every spot-check measurement to verify the quality and reliability of the ventricular rate recordings. Similar diligence is recommended when applying these protocols.

## Study limitations

This study has several limitations including a relatively small population size and a bias for inadequate VRR control. The chosen treatments of underlying heart disease, arrhythmia or congestive heart failure were not standardised and were based on clinician judgement. This may have had an impact on population selection towards cases deemed sufficiently stable to warrant an aECG. However, this in itself would not affect the agreement observed between the spot-check protocols and the aECG data.

The usefulness of the spot-check protocols was not tested against patient outcome data, and a specific cut-off for the protocols was not set. Instead, these were assessed by comparing their ability to identify dogs with adequate rate control of AF based on cut-offs designed for aECG data that had been previously demonstrated to predict survival [5,6,9]. A cut-off that predicted adequate rate control (as opposed to inadequate rate control) was not confidently established because insufficient dogs had aECG rates <125 bpm to allow robust analysis. Despite efforts to maximise case inclusion, including accepting of multiple recordings per dog, the number of cases with optimal rate control (<125 bpm) remained limited. This reflects the constraints of case availability inherent to clinical referral-based research.

**Table 3** Mean and mesor ventricular response rates (VRRs) in individual dogs obtained from ambulatory ECG and mean and median VRRs obtained from three spot-check protocols. Protocol A: three spot-checks measured eight h apart; protocol B: three spot-checks measured six h apart during daytime; protocol C: five spot-checks. Data from each protocol used eight-hourly or six-hourly measurements and are displayed as median and mean values. Patients with VRRs suggestive of adequate rate control and their measurements (<125 bpm [5,6]) are highlighted in green.

| | Ambulatory ECG data | | Protocol A | | Protocol B | | Protocol C | |
|---|---|---|---|---|---|---|---|---|
| Patient | Mean | Mesor | Median AliveCor® | Mean AliveCor® | Median AliveCor® | Mean AliveCor® | Median AliveCor® | Mean AliveCor® |
| 1 | 177 | 169 | 195 | 199 | 196 | 199 | 196 | 195 |
| 2 | 180 | | 222 | 200 | 218 | 201 | 196 | 218 |
| 3 | **109** | **98** | **117** | 131 | 144 | 148 | 145 | 144 |
| **4** | 161 | 157 | 164 | 166 | 165 | 168 | 164 | 164 |
| 5 | 157 | 175 | 236 | 247 | 205 | 222 | 223 | 205 |
| 6 | 166 | 166 | 163 | 167 | 171 | 166 | 165 | 163 |
| 7 | 150 | 150 | 165 | 173 | 155 | 141 | 145 | 146 |
| 8 | **72** | **72** | **110** | **107** | **110** | **107** | 158 | 155 |
| **9** | **96** | **100** | **90** | **95** | **90** | **93** | **106** | **110** |
| **10** | **111** | **111** | **125** | **125** | 140 | 132 | **95** | **90** |
| **11** | 158 | 158 | 197 | 189 | 194 | 192 | 126 | 127 |
| 12 | 184 | 190 | 209 | 209 | 206 | 202 | 189 | 194 |
| 13 | 158 | 158 | 184 | 177 | 170 | 160 | 205 | 209 |
| 14 | 166 | 166 | 238 | 236 | 241 | 238 | 170 | 160 |

The negative and positive predictive values of a test are dependent on the prevalence of disease (in this case, the number of dogs with inadequate VRR), which in this population was high and may not reflect the general population.

Regarding quality of aECG recordings, the R-R intervals were visually inspected but oversensing or undersensing of R waves was still possible, given the inability to manually correct these. However, by including examinations with <10% artifact, the impact of artifact and undersensing was minimised. This dependence on the quality of the recording is a recurrent problem with clinical cases, thus reflecting the clinical setting.

Timing the spot-checks to match the exact same time point on the aECG (accurate to milliseconds) was not feasible and therefore not attempted. Ambulatory ECG data are recorded in milliseconds, but this level of rigour was not possible in the home environment.

It is possible that the process of measuring VRR by use of a smartphone-based ECG device influenced mean VRR. Despite all efforts to reduce the stress during the measurements, it is possible that dogs that would otherwise be asleep or resting became excited by the contact with the owner, biasing their results of mean, median and mesor VRR. Attempts were made to reduce this effect by allowing a degree of acclimatisation of the dog to the device before readings. Nonetheless, there is a high agreement of these measurements of VRR taken over 30 s with averages taken over one

**Table 4** Sensitivity, specificity, positive and negative predictive values calculated from the mean ventricular response rate (VRR) obtained with three spot-check protocols identifying canine patients with suboptimal control of the VRR in atrial fibrillation (defined as mean VRR >125 bpm [5,6] and VRR >140 bpm [9]). Sensitivity, specificity, positive and negative predictive values and accuracy are shown as percentage values. Protocol A: three spot-checks measured eight h apart; protocol B: three spot-checks measured six h apart during daytime; protocol C: five spot-checks.

| Mean VRR from 24 h ECG | Parameter | Protocol A | Protocol B | Protocol C |
|---|---|---|---|---|
| >125 bpm | Sensitivity and CI (%) | 90 (74.1—100) | 100 (74.1—100) | 100 (74.1—100) |
| | Specificity and CI (%) | 75 (19.5—99.4) | 50 (6.6—93.2) | 50 (6.6—93.2) |
| | PPV and CI (%) | 90.9 (58.7—99.8) | 83.3 (51.6—97.9) | 83.3 (51.6—97.9) |
| | NPV and CI (%) | 100 (36.8—100) | 100 (22.4—100) | 100 (22.4—100) |
| | Accuracy and CI (%) | 92.9 (66.1—99.8) | 85.7 (57.2—98.2) | 85.7 (57.2—98.2) |
| >140 bpm | Sensitivity and CI (%) | 100 (74.1—100) | 100 (74.1—100) | 90 (55.6—99.7) |
| | Specificity and CI (%) | 100 (47.3—100) | 75 (19.4—99.4) | 50 (6.6—93.2) |
| | PPV and CI (%) | 100 (74.1—100) | 90.9 (58.7—99.8) | 81.8 (48.2—97.7) |
| | NPV and CI (%) | 100 (47.3—100) | 100 (36.8—100) | 66.7 (9.4—99.2) |
| | Accuracy and CI (%) | 100 (80.1—100) | 92.9 (66.1—99.8) | 78.6 (49.2—95.3) |

Bpm: beats per minute; CI: confidence interval; ECG: electrocardiogram; NPV: negative predictive value; PPV: positive predictive value; VRR: ventricular response rate.

whole hour, when the dogs would have had time to recover from the initial measurement. This implies that the VRR increase was not as important as one might expect.

Finally, it is also worth mentioning that spot-check protocols are specifically designed for assessing VRR in dogs with AF and not for identifying concomitant ventricular arrhythmias or assessing their circadian variation [3]. Therefore, they cannot completely replace aECG studies. In this context, believe they complement aECG and possibly reduce the number of aECG that would be appropriate for any dog with AF.

## Declaration of Competing Interest

The authors do not have any conflicts of interest to disclose.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jvc.2025.10.002.

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