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Diagnostic value of linear and non-linear analyses of heart rate variability for differentiation of homeostasis in health status, disease state, or death in dogs

[Valor diagnóstico de análises lineares e não lineares da variabilidade da frequência cardíaca para diferenciação da homeostase em estado de saúde, doença e risco de óbito em cães]

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ABSTRACT

The autonomic nervous system is closely linked to heart rate and is characterized as a chaotic deterministic system. As the heart is therefore controlled by a non-linear system, these analyzes are being used more and more. The aim of this study was to evaluate nonlinear indices of recurrence graphs and linear indices in healthy, sick and at risk of death dogs and to demonstrate whether these indices have good diagnostic accuracy to identify dogs at risk of death. Sixty-six dogs underwent heart rate variability analysis using a frequency meter. The results showed that the SDNN, RMSSD, PNN50% and DET% indices showed a significant difference between all groups, helping to differentiate the health status in terms of autonomic homeostasis. Greater sensitivity (96.67%) was observed for linear indices (SDNN, RMSSD and PNN50%) and greater specificity (100%) for non-linear indices (DET%, REC% and LAM%) in the recognition of dogs at risk of death. Linear indices (SDNN, RMSSD and PNN50%) and non-linear indices (DET% and ShanEnt) showed greater diagnostic accuracy for identifying healthy or dying dogs. It is concluded that the studied indices of heart rate variability help to differentiate the health status in healthy dogs and are excellent predictors of prognosis.

Keywords: Autonomic nervous system, Heart rate variability, Heart rate monitor, Chaos theory, canine

RESUMO

O sistema nervoso autônomo está intimamente ligado à frequência cardíaca e se caracteriza como um sistema determinístico caótico. Como o coração é, portanto, controlado por um sistema não linear, essas análises estão sendo cada vez mais usadas. O objetivo deste estudo foi avaliar os índices não lineares dos gráficos de recorrência e os índices lineares em cães saudáveis, doentes e com risco de morte e demonstrar se esses índices apresentam boa acurácia diagnóstica para identificar cães em risco de morte. Sessenta e seis cães foram submetidos à análise da variabilidade da frequência cardíaca por um frequencímetro. Os resultados mostraram que os índices SDNN, RMSSD, PNN50% e DET% apresentaram diferença significativa entre todos os grupos, auxiliando na diferenciação do estado de saúde em termos de homeostase autonômica. Foi observada maior sensibilidade (96,67%) para índices lineares (SDNN, RMSSD e PNN50%) e maior especificidade (100%) para índices não lineares (DET%, REC% e LAM%) no reconhecimento de cães em risco de morte. Índices lineares (SDNN, RMSSD e PNN50%) e índices não lineares (DET% e ShanEnt) apresentaram maior acurácia diagnóstica para identificar cães saudáveis ou em risco de morte. Conclui-se que os índices estudados da variabilidade da frequência cardíaca auxiliam na diferenciação do estado de saúde em cães saudáveis e são excelentes preditores de prognóstico.

Palavras-chave: sistema nervoso autônomo, variabilidade da frequência cardíaca, monitor de frequência cardíaca, teoria do caos, cães

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INTRODUCTION

Chaos is a dynamic system that seems random, although it has an underlying order. Some degree of chaos is universally present in living systems, and several components with chaotic patterns have already been detected, such as the anatomical distribution of venous and bronchial branches since blood vessels do not form a perfect chain but rather a branch of the network. Another example would be cardiac electrical activity because the pattern of an electrocardiogram reveals a multitude of peaks and inclinations that vary in time, according to heartbeat variability (Korolj *et al.*, 2019).

Most existing systems in nature must behave as a deterministic nonlinear dynamic complex system, that is, a system that changes over time in a causal way. Its future depends only on phenomena of its past and present, where every event is physically determined by an uninterrupted chain of previous occurrences; therefore, these systems must obey chaos theory (Oestreicher, 2007).

Living organisms are certainly a system with all the abovementioned characteristics, in which chaos would have a positive concept reflecting the health situation; in other words, the organism is prepared to respond favorably to the aggressions of the environment, providing all its capacity. In clinical situations, great variability is observed in the terminal conditions that depend sensitively on the initial condition. Thus, small dysfunctions in isolated organs gradually lead to certain degrees of dysfunction as they associate in a gradual manner and sometimes result in catastrophic situations. When chaos is absent, continuous changes in life lead to states of disease until the occurrence of balance and, as a result, death (Selig *et al*., 2011).

The autonomic nervous system (ANS) is closely linked to the heart rate (HR), which undergoes physiological variations in each heartbeat. This control mechanism occurs by vagal and medullary afferent pathways, by which the information that reaches the central nervous system (CNS) is modulated and returns to the heart by slow sympathetic and rapid vagal efferent fibers. As the uptake of norepinephrine released in sympathetic terminations is slower than that of acetylcholine in parasympathetic terminations, this difference observed in the rate of transmission in cholinergic and adrenergic pathways will result in inequalities in the modulation frequency of these two systems in the sinus node (Sá *et al*., 2013).

Changes in heart rate may be habitual, for example, in exercises versus resting states, and under other autonomic influences. The variability of the time intervals between heartbeats is called heart rate variability (HRV). Natural HRV has been shown to be a chaotic deterministic system. Any changes in HRV and its fractal properties were distinctly recognized because they were related to the pathophysiology of the disease. This has been especially useful for its predictive and diagnostic value, with the general rule being that reduced variability is a characteristic of the disease (Korolj *et al.*, 2019).

There is increasing interest in evaluating the complexity of cardiovascular control by analyzing short-term heart rate variability; this is an analysis of the activity of vasomotor and respiratory centers, baroreflex and chemoreflex regulation, mediated by vagal and sympathetic interferences. All these mechanisms act on similar frequencies but are not coincidental. The joint action of these mechanisms or the dominant action of one of these mechanisms determines the reduction of complexity, that is, the increase in regularity and predictability, which is considered a marker of morbidity (Porta *et al*., 2001).

Human cardiac monitors are already used in animal studies to assess HRV in the short term. The measurement of RR intervals performed through the Polar®[c] monitor is already a validated method for dogs (Jonckheer-Sheehy *et al*., 2012; Essner *et al*., 2013; Martinello *et al*., 2022).

Because the heart is controlled by a nonlinear deterministic system, nonlinear dynamics measurements are increasingly employed. Recurrence plots (RP) can analyze recurrences in the dynamics of a system and can also display specific patterns of large and small scales, which are produced by typical dynamic behavior, for example, diagonal lines (temporal evolution, similar location of different parts of the trajectory) or vertical and horizontal lines (the state does not change for a time). The structures within the RPs can be described by the analysis of recurrence quantification, which is an appropriate method for the analysis of short sequences and nonstationary RR intervals; additionally, this recurrence quantification is sensitive to small changes in system dynamics (Javorka *et al*., 2008; Dimitriev *et al*., 2020).

RPs, designed by Eckmann *et al*., in 1987, were proposed to investigate the behavior of systems characterized by time series in an abstract space called the phase space which is a highly effective and widely accepted tool to study time series. Its formation is demonstrated in a square in which both the x-axis and the y-axis contain the elements of the time series, arranged sequentially from the first to the last, correlating every two elements. Thereafter, according to pre-established parameters (dimension, delay, and radius), the recurrence of values can be analyzed. The different colors represent different rays, and rays with equal distances have the same color; this complements the visual appearance and characteristics of the graph (Takakura *et al*., 2017).

A study of RPs conducted in humans of various age groups (newborns and healthy children, healthy young and middle-aged adults, elderly, people with advanced chronic kidney disease and people with brain death or imminent death) described quantitative and qualitative aspects that allow differentiation between health states, advanced disease states and death or imminent death throughout life. In this study, the most relevant indexes were TT trapping time (nonlinear measurement) and SDNN (linear measurement) (Godoy and Gregory., 2019).

To date, there are no studies evaluating HRV in healthy, sick, and life-threatening dogs analyzed by recurrence charts. It is assumed that detailed knowledge about cardiac autonomic homeostasis in healthy individuals and patients by nonlinear methods allows a better understanding of this complex system. Thus, the aim of this study was to evaluate the indexes of recurrence charts and linear indexes in healthy and sick dogs and those at risk of death and to demonstrate whether these indexes present good diagnostic accuracy to identify dogs at risk of death.

MATERIALS AND METHODS

The study was conducted in accordance with animal welfare standards after approval by the Committee for Ethics in the Use of Animals of the Faculty of Veterinary Medicine of UNESP - FMVZ - Botucatu (CEUA protocol n° 204/2017) and after the written consent form by tutors. This was a prospective study with 66 dogs. Thirty-six males and 30 females of different breeds and sizes were divided into three groups. Group 1 (control group) was composed of 30 young adult dogs, group 2 was composed of 14 dogs with chronic diseases, and group 3 was composed of 22 dogs at risk of death.

All dogs underwent a previous physical examination (heart rate, respiratory rate, rectal temperature), followed by a specific cardiovascular physical examination (mucosal staining, capillary refill time, auscultation of all cardiac foci, assessment of precordial shock, degree of hydration and femoral pulse), echocardiographic and electrocardiographic examination. Inclusion criteria in group 1 were healthy animals, with no changes in physical, echocardiographic and/or electrocardiographic examinations, between one and eight years of age. This age was chosen due to the result of a previous study, which reported that patients at this age present better HRV (Martinello *et al*., 2022). Group 2 included dogs with clinically stable chronic diseases (asymptomatic patients with controlled underlying disease), and group 3 included only dogs that died within 15 days after analysis with the frequency meter. On the other hand, the exclusion criteria for group 1 were brachycephalic animals and any dog that presented any previous cardiac alteration. In group 2, dogs with decompensated underlying diseases were excluded, and in group 3, animals that died after 15 days of analysis with the frequency meter were excluded. All dogs that presented arrhythmia were excluded.

Electrocardiographic data (1,000 consecutive RR intervals) were digitally captured in a Polar device, model® V-800, already validated for animals for this purpose (Marchant-Forde *et al*., 2004; Jonckheer-Sheehy *et al*., 2012; Essner *et al*., 2013; Paula *et al*., 2019; Martinello *et al*., 2022). First, an electrocardiogram was performed to visualize possible arrhythmias. Then, the heart rate monitor was performed after trichotomy of the area for better electrode contact and less interference. After that, the electrodes were placed on each side in the sternal region at the same height as the fourth intercostal space, as performed in human neonates (Selig *et al*., 2011). The device was connected in two adhesive pediatric electrodes, thus without the elastic strap, and fixed next to the animal's body, which was aided using adhesive adhesives and bandages. The dogs were allocated to a room with their owners for 15 minutes to adapt to the environment; then, the recording was performed. The dogs were released in this same room only with their owners to perform the exam, and the devices were only switched off at the end of a period of five to eight minutes. The heart rate monitor used recorded the R-R intervals for the same period. Subsequently, the R-R intervals were transferred to a computer for analysis. These intervals were digitally filtered to eliminate premature beats and noise and then manually reanalyzed to exclude residual artifacts. Only time series with less than 10% artifacts were included for analysis. The linear indexes of the study were the mean of RR intervals (mean RR), standard deviation of the mean of normal RR intervals (SDNN), the square root of the mean square of the differences between adjacent RR intervals (RMSDD), and the percentage of adjacent RR intervals with duration differences greater than 50 ms (PNN50%).

The nonlinear indexes were evaluated by the recurrence plots, and the following quantitative indexes were obtained: percentage of recurrence points in the phase plane (recurrence rate; REC%), percentage of recurrence points forming diagonal lines (determinism; DET%), percentage of recurrence points forming vertical lines (laminarity; LAM%), average length of vertical lines (imprisonment time; TT) and Shannon entropy (a measure that indicates the complexity of the system; ShanEntr). Visual Recurrence Analysis (VRA - Version 5.01, Eugene Kononov, http://visual-recurrence-analysis.software.informer.com) software was used for these analyses. Recurrence charts were constructed with the following parameters: dimension = 2, delay = 2, radius = 70, line = 2) and Volcano (as color scheme) [13], and Kubios® software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

Normal distribution was evaluated using the Shapiro–Wilk test. The comparison of the means/medians of the variables between the groups was performed by the t test for independent samples (when a normal distribution occurred) or the Mann–Whitney nonparametric test (when a normal distribution did not occur). For the comparison of the mean/median between all groups, ANOVA (when the assumption of normality was met) and the Kruskal–Wallis test (when the assumption of normality was not met) were used. Multiple comparisons were applied to detect which groups differed from each other.

The efficacy of the discrimination of the variables for groups 2 and 3 against the control group was performed by calculating sensitivity, specificity, positive predictive value, negative predictive value, and accuracy (with the respective confidence intervals) and constructing the ROC curve. The area under the curve (AUC) values were used to choose the most efficient variables (AUC above 80%). Descriptive analysis of all quantitative variables, such as the mean, standard deviation, and quartiles for each group, was performed. A p value lower than 0.05 was considered to indicate a significant difference between the respective groups.

RESULTS

This was a prospective study using 66 dogs. 36 males and 30 females of various breeds were divided into three groups. Group G1 (control) had 30 dogs, 14 males and 16 females, with a mean age between 3.61±1.93 years old and an average weight of 8.8±2.52kg.

Group G2 (sick dogs) was composed of 14 dogs, 10 males and four females, with a mean age between 12.07±2.32 years old and an average weight of 7.56±2.66kg. In this group, dogs had different chronic clinical conditions, such as stage C mitral valve myxomatous disease [ACVIM Consensus – 2019] (4/14), chronic renal disease in stage III [International Renal Interest Society, International Society of Renal Interest] (5/14) and dogs with diabetes mellitus, pulmonary neoplasia, liver neoplasia, chronic pancreatitis and cardiorenal syndrome (1/14 each), totaling 14 animals. Group G3 (risk of death) was composed of 22 dogs that died 6.69±6.07 days after analysis with the frequency meter, 12 males and 10 females, with a mean age between 11.84±2.33 years and a mean weight of 6.09±2.88 kg. In this group, eight animals (8/22) had end-stage neoplasia (with multicenter lymphoma [1/8], cutaneous mastocytoma [2/8], pulmonary carcinoma [3/8], renal carcinoma and renal-based neoformation generating obstruction in the cranial vena cava [2/8]); six dogs (6/22) had stage D mitral valve myxomatous disease [ACVIM Consensus – 2019], five dogs (5/22) had stage IV chronic kidney disease [International Renal Interest Society]; two dogs (2/22) had refractory pneumonia; and one dog (1/22) epilepsy.

HRV differed significantly between groups ($p<0.05$) in different clinical states (G1, G2 and G3). Healthy dogs (G1) presented linear HRV indexes (RR, SDNN, RMSSD and PNN50%) that were significantly higher than those of the other groups (G2 and G3) (Tables 1 and 2). The only nonlinear index that showed a significant difference between healthy dogs (G1) and sick dogs (G2) was determinism (DET%). The recurrence rate (REC%), determinism (DET), laminarity (LAM%), time of imprisonment (TT) and entropy of Shannon index (ShanEnt) showed a significant difference ($p<0.05$) between healthy dogs (G1) and dogs at risk of death (G3) (Table 1). In the comparison between groups G2 and G3, there was a significant difference ($p<0.05$) between all indexes studied, except for the mean RR. The standard deviations, means and medians of each index are presented in Table 1. The significant differences are highlighted in bold between the analyzed groups ($p<0.05$) (Table 2).

The SDNN, RMSSD, PNN50% and %DET indexes showed a significant difference between all groups ($p<0.05$). The nonlinear indexes REC%, %LAM, TT and ShanEnt showed no significant difference between groups G1 and G2, which made it impossible to distinguish the clinical states between patients and healthy patients.

The animals of group 3 (risk of death) presented significantly lower values of all indexes in the time domain and significantly higher values of nonlinear indexes than animals in group 1 (healthy dogs).

Table 1. Mean ± standard deviation and [median] of linear (RR, SDNN, RMSSD and PNN50%) and nonlinear (REC, DET, LAM, TT, ShanEnt) indexes of HRV in dogs in the different groups analyzed

Indexes	Control group (n = 30) G1	Dogs with diseases chronicles (n = 14) G2	Dogs at risk of death (n = 22) G3
Interval RR	580.57±111.74[a] [542.57]	463.92±95.63[bc] [437.5]	379.18±79.94[c] [371]
SDNN (msec)	71.74±24.90 [a] [72.15]	33.28±11.740[b] [32.1]	12.63±8,968[c] [9.7]
RMSSD (msec)	93.04±40.28 [a] [85.9]	32.86±18.36[b] [27.4]	6.68±4.980[c] [4.8]
PNN50%	52.56±19.42 [a] [57.00]	14.29±15.02[b] [7.06]	0.340±0.782[c] [0]
REC%	32.27±1.858 [a] [31.71]	31.56±6.314[ab] [32.60]	38.73±4.994[c] [40.462]
DET%	52.08±8.798 [a] [48.54]	66.55±1 6.90[b] [62.64]	87.21±15.54[c] [93.13]
LAM%	60.96±12.41[a] [61.67]	73.87±14.84[ab] [72.68]	90.87±10.79[c] [95.24]
TT	3.216±0.727 [a] [2.999]	4.591±3.051 [ab] [3,574]	11.41±6.46[c] [10.64]
Shanent Bits	2.465±0.272 [a] [2.408]	2.952±0.663 [ab] [2,930]	4.156±0.778[c] [4.216]

RR interval (mean normal RR intervals); SDNN (standard deviation of the mean of normal RR intervals); RMSSD (square root of the mean sum of the square difference of adjacent normal RR intervals of the whole exam), PNN50% (percentage of difference greater than 50 msec between adjacent normal RR intervals), REC% (the percentage of recurrence points in a recurrence chart); DET% (the percentage of recurrence points forming diagonal lines); LAM% (the percentage of recurrence points forming vertical lines); TT (the average length of vertical lines); ShanEntr (Shannon entropy: a measure that indicates the complexity of the system). Different letters in the same line represent significant differences between the groups (*one-way ANOVA* and Tukey–Kramer; $p<0.05$)

Table 2. Comparison between groups (G1 - control), G2 (chronic diseases) and G3 (dogs at risk of death) and statistical significance ($p<0.05$)

Groups	interval RR	SDNN (msec)	RMSSD (msec)	PNN50%	REC%	DET%	LAM%	TT	Shanent Bits
G1XG2	0.0336	0.0029	0.0057	0.0048	1,0000	0.0324	0.0716	0.3833	0.1169
G1XG3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G2XG3	0.0841	0.0273	0.0112	0.0129	0.0004	0.0303	0.0237	0.0034	0.0046

The time domain indexes, namely, SDNN, PNN50% and RMSSD, were more sensitive (96.67%), with a low false-negative rate, and nonlinear rates (DET%, REC% and LAM%) were more specific (100%), presenting a low rate of false positives in the recognition of dogs at risk of death. Greater diagnostic accuracy was obtained for the RMSSD (99.8%), PNN50% (99.5%), SDNN (99.4%), ShanEnt (98.4%) and DET% (96.4%) indexes.

The AUCs of the linear and nonlinear indexes more predictive of the risk of death (SDNN, PNN50%, RMSSD, DET % and ShanEnt) overlapped; they did not totally differ and crossed at certain points, indicating little difference between them (Fig. 1).

The sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy, and cutoff of each index are presented in Table 3.

In the plots of recurrence analyses (qualitative analysis) of the most representative cases of each group analyzed, it can be observed that the animals in group 1 presented a more diffuse and homogeneous distribution when compared to groups 2 and 3, which exhibited more geometric patterns. The lower the HRV was, the more geometric and linear the graph was presented (Fig. 2).

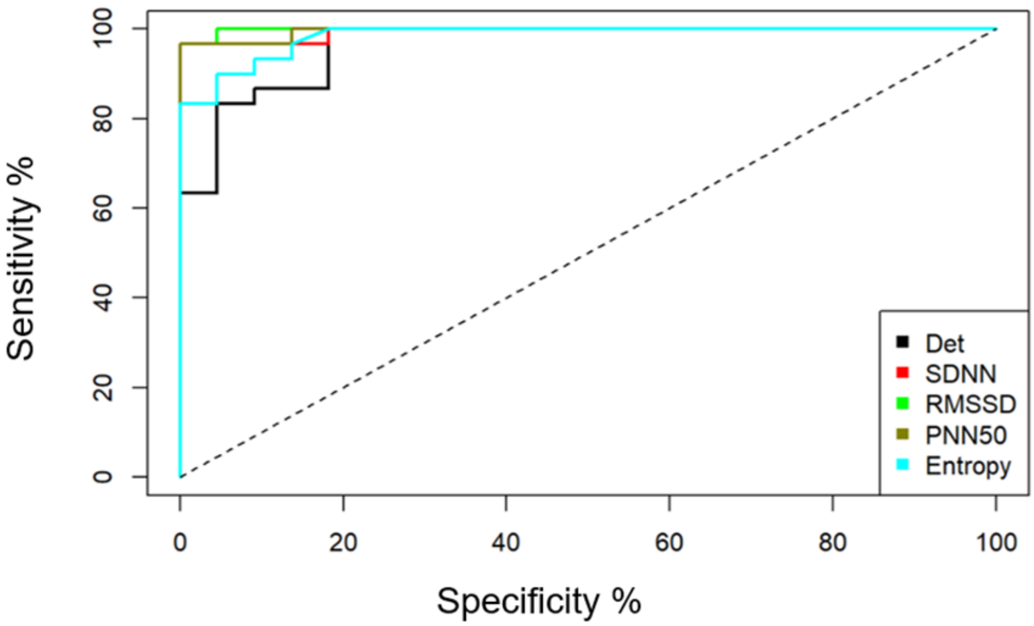

Figure 1. *Receiver operating curves* of the most diagnostic accuracy indexes for the risk of death in patients. SDNN (standard deviation of the mean of normal RR intervals); RMSSD (square root of the sum mean of the difference of squares of adjacent normal RR intervals of the whole exam); PNN50% (percentage of difference greater than 50 msec between adjacent normal RR intervals); DET% (percentage of determinism); Entropy/ShanEntr (Shannon entropy).

Table 3. *Receiver operating characteristics* of linear HRV indexes (RR; SDNN, RMSSD and PNN50) and nonlinear (REC, DET, LAM, TT and ShanEnt), diagnostic accuracy and cutoff for dogs at risk of death

Indexes	SE for risk of death, % (95% CI)	SP for risk of death, % (95% CI)	PPV for risk of death, % (95% CI)	NPV for risk of death, % (95% CI)	Accuracy, % (95% CI)	Cutoff
interval RR	81.82 (59.72-94,81)	93.33 (77.93-99.18)	90.00 (68.30-98.77)	87.50 (71.01-96.49)	92.6 (85.1-100.2)	< 453 ms > RM
SDNN	95.45 (77.19-99.88)	96.67 (82.78-99.92)	95,45 (77.16-99.88)	96.67 (82.73-99.92)	99.4 (98.1-100.7) *	< 34.7 ms >RM*
RMSSD	95.45 (77.19-99.88)	96.67 (82.78-99.92)	95.45 (77.16-99.88)	96.67 (82.73-99.92)	99.8 (94.4-100.3)*	< 19.0 ms >RM*
PNN50	95.45 (77.19-99.88)	96.67 (82.78-99.92)	95.45 (77.16-99.88)	96.67 (82.73-99.92)	99.5 (98.5-100.6)*	< 2.54% >RM*
Rec	68.18 (45.13-86.14)	100.0 (88.43-100.0)	100.0 (78.20-100.0)	81.08 (64.84-92.04)	90.8 (80.9-100.6)	>39.12% > RM
Detective	77.27 (54.63-92.18)	100.0 (88.43-100.0)	100.0 (80.49-100.0)	85.71 (69.74-95.19)	96.4 (92.0-100.7)*	>87.75% > RM*
Lam	77.27 (54.63-92.18)	100.0 (88.43-100.0)	100.0 (80.49-100.0)	85.71 (69.74-99.19)	95.3 (90.1-100.5)	>87.34% > RM
Tt	81.82 (59.72-94.81)	93.33 (77.93-99.19)	90.00 (63.30-98.77)	87.50 (71.01-96.49)	95.3 (90.1-100.5)	>4.66% > RM
Shanent	90.91 (70.84-98.8)	90.00 (73.47-97.89)	86.96 (66.41-97.22)	90.9 (78.97-96.80)	98.4 (96.1-100.7)*	> 2.86% > RM*

Sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), accuracy and cutoff of each index were evaluated. Sensibility for risk of death, % (CI95%) Specificity for risk of death, % (95% CI%) for risk of death, % (95% CI%) NPV for risk of death, % (95% CI%) Accuracy, % (CI95%). Risk of death (MR) and cutoff values < (lower) or > (higher)

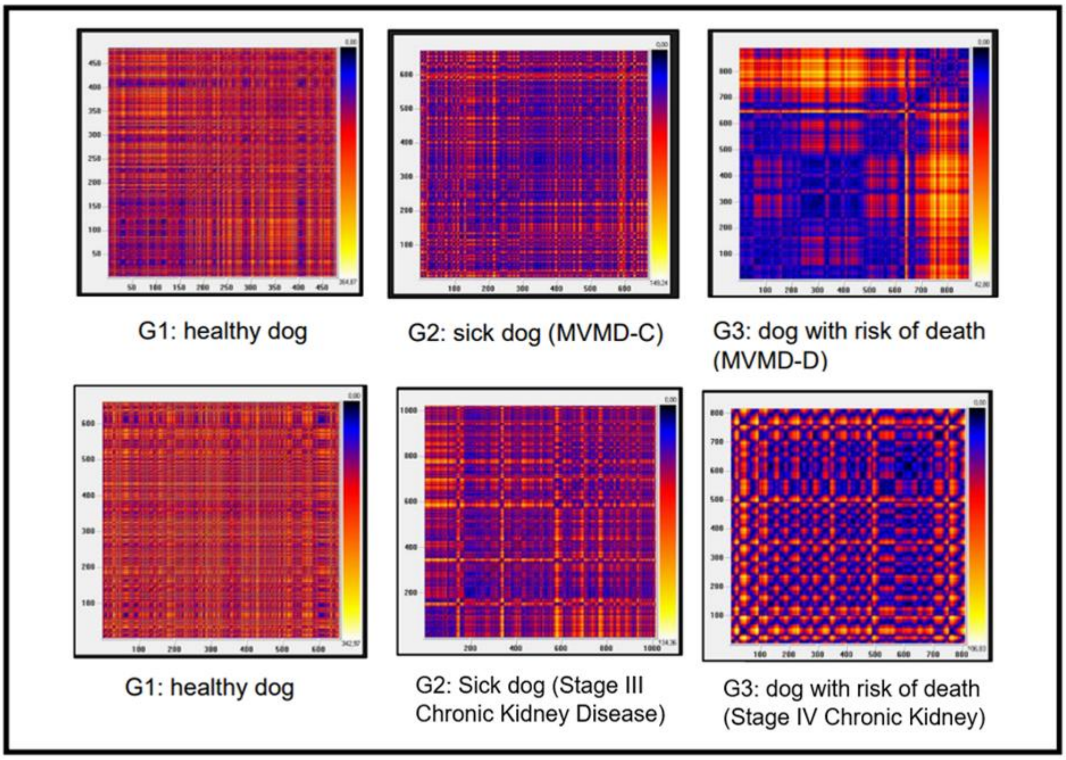

Figure 2: Representative recurrence plots of dogs chosen to represent each of the groups: G1- control group, G2: dogs with chronic diseases (Stage C mitral myxomatous degenerative disease; Stage III Chronic Kidney Disease) and G3- death-risk dogs (Stage D mitral myxomatous degenerative disease; Stage IV Chronic Kidney Disease).

Comparisons between sick and life-threatening dogs showed significantly lower SDNN (p=0.0273) in group G3 compared to group G2; however, the RR interval did not differ significantly (p=0.0841) between these groups, showing that the difference in HRV is not only associated with changes in heart rate.

DISCUSSION

The present study aimed to describe the indexes of recurrence plots and linear indexes in healthy dogs, sick patients and at risk of death and to characterize the cardiac autonomic behavior of these groups by evaluating whether these indexes present good diagnostic accuracy regarding the risk of death in dogs.

It was observed in this study that HRV decreases according to health status, increasing in morbid states and at risk of death. Linear and nonlinear indexes have been shown to be useful for assessing clinical status and autonomic homeostasis in dogs.

Cardiac homeostasis depends on the dynamic balance between the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The control of cardiac homeostasis occurs mainly through sympathetic and parasympathetic endings throughout the heart, which directly interfere with heart rate values and beat-to-beat variability. Thus, the loss or reduction in HRV indirectly reflects a reduction in chaotic behavior, which can translate into compromised homeostasis. For this reason, groups 2 and 3 were composed of animals with different diseases, as the pathological state generates high levels of circulating catecholamines that cause deleterious effects, leading to increased sympathetic activity and decreased parasympathetic activity, which in turn decreases. the VFC. HRV is the result of the regulation of the autonomic nervous system, which is affected by any type of disease (Kleiger *et al*., 2005; Vanderlei *et al*., 2009; Godoy and Gregory, 2019). Corroborating a human study based on an extensive amount of data published in the literature, it joined in the same group several individuals with different diseases and showed that these people had low HRV (Godoy and Gregório., 2022).

In group 1, brachycephalic dogs were excluded because their morphology makes these dogs present a greater inspiratory effort, increasing the parasympathetic tonus and physiologically increasing their HRV (Trauffler *et al*., 2019).

In the present study, HRV analysis was performed for five to eight minutes with dogs without sedation. All indexes analyzed showed significant differences in the comparison of at least one group, indicating that short-term analysis with the heart rate monitor is feasible to assume the clinical and autonomic state of a patient. The evaluation performed in a short period presents fewer ectopia events or artifacts that interfere in the analysis of HRV, which may compromise the reliability of the results obtained (Marchant-Forde *et al*., 2004). Corroborating the present study, a study conducted with 79 dogs from different age groups performed HRV analysis over five minutes and concluded that HRV derived from electrocardiographic recording over five minutes in clinical settings is a reliable method to evaluate the function of the autonomic nervous system [20]. HRV performed in a short period is an acceptable analysis because in a few minutes, we can obtain the results; furthermore, it is possible to evaluate the cardiac autonomic behavior of healthy and sick dogs. Several studies have obtained satisfactory results in the evaluation of HRV in a short period (Piccirillo *et al*., 2009; Katayama *et al*., 2016; Bogucki e Noszczyk-Nowak, 2017; Maccariello *et al*., 2018).

In the present analysis, we chose to use the heart rate monitor because the aim was to analyze HRV in dogs with less stress possible in the clinical routine. The HR monitor proved to be a great option for HRV analysis because it was possible to record RR intervals with less stressed patients and to transfer these results to diverse HRV analysis software available on the market, such as [d]Recurrence Analysis and [e]Kubios®. The heart rate monitor is a noninvasive and financially accessible method for HRV analysis and is advantageous in relation to electrocardiography because it causes less discomfort to the patient, with a good level of agreement between these two methods in dogs and human beings (Essner *et al*., 2013, 2015; Barbosa *et al*., 2016).

HRV indexes in the time domain are the most studied in the veterinary cardiology routine; the SDNN is correlated with the sympathetic and parasympathetic components of the autonomic nervous system, whereas RMSSD and PNN50% consider short and rapid changes between adjacent RR intervals, based on the comparison between the duration of two adjacent cycles, which allows the evaluation of parasympathetic vagal tone (Marães, 2010). However, to the authors' knowledge, there are no publications that evaluated the measures of the recurrence chart in healthy dogs, sick patients, and those at risk of death. Nonlinear measures, such as REC%, ShanEn, LAM%, TT and DET%, were proposed as measures of complexity, and smaller values indicate greater complexity of system dynamics (Javorka *et al*., 2008).

In the present study, group 1 (control group) was formed by healthy young adult dogs (3.61±1.93 years old), who present autonomic behavior more compatible with homeostasis, that is, considered chaotic; this group presented higher HRV, with higher rates of SDNN, RMSSD and PNN50% and lower recurrence indicators, demonstrating that this group presented better functioning of the ANS. In a previously performed analysis that evaluated HRV in the time and frequency domain in 87 dogs of different age groups for 20 minutes, dogs between 1.1 and 7.9 years of age presented higher HRV than puppies (<1 year) and elderly dogs (>8 years) (Martinello *et al*., 2022).

Dogs in groups 2 and 3 presented lower rates of SDNN, RMSSD and PNN50% and higher recurrence indicators, which demonstrates deterioration of ANS homeostasis in these groups, so aging is not the only factor that leads to loss of system complexity but also the presence of a disease.

The linear indexes were significantly lower ($p<0.05$) in animals at risk of death than in sick animals, and all analyses of recurrence quantification were significantly higher in dogs at risk of death than in dogs with chronic diseases ($p<0.05$), resulting in lower variation and, therefore, greater autonomic impairment. Animals with chronic disease already present increased sympathetic activity and decreased parasympathetic activity, with a subsequent decrease in HRV, with inadequate treatment or no treatment at all, a greater balance of the system (absence of chaos) and, as a result, death.

The time domain analysis and the nonlinear index (%DET) were higher in the comparison between healthy and sick dogs because group G1 presented significantly higher values in the indexes of the time domain and a lower value of the %DET index. A human study evaluated recurrence rates in 17 young patients with diabetes mellitus [9], revealing significantly higher values of %REC and TT in these young people than in the control group (healthy patients), which reveals changes in dynamic recurrences with reduced HR complexity. In the present study, higher TT and % REC indexes were demonstrated in sick patients, but there was no significant difference between these groups. HRV is the result of the regulation of the autonomic nervous system, which is affected by any type of condition; the SNS (sympathetic nervous system) is one of the two branches of the nervous system that is involved in the regulation of numerous homeostatic mechanisms, including cardiac function. The main function of the SNS is to stimulate the fight or flight response of the body, but in morbid states, high circulating catecholamine levels are generated, which causes harmful deleterious effects (Kleiger *et al*., 2005; Selig *et al*., 2011; Tarvainen *et al*., 2014; Lucia *et al*., 2018).

All indexes studied showed significant differences between groups 1 and 3, which shows that all indexes studied are useful for the differentiation between these two groups. Dogs at risk of death presented a very low HRV, exhibiting a decreased parasympathetic tone (SDNN, RMSSD and PNN50% with extremely low values) and presented higher recurrence and predictability (%REC, %DET, TT, LAM% and ShanEn), which indicates progression toward the equilibrium state and detachment from chaotic behavior; young adult dogs, on the other hand, went in the opposite direction because they presented a normal HRV, lower recurrence and less predictability because they were healthy individuals considered chaotic.

In the qualitative analysis of recurrence plots, a more diffuse and homogeneous distribution was observed in healthy young adult dogs, reinforcing that this group of patients presented higher HRV, less predictability, less recurrence, and more chaos. In groups 2 and 3, more geometric patterns were observed, and in group 3, there was a greater number of large squares, visibly showing that these patients present a loss of complexity and simplification of dynamic heart rate in pathological conditions, a fact observed and described in humans in recurrence plots (Godoy and Gregory, 2019).

The ability of linear and nonlinear indexes to discriminate patients at risk of death against various diseases was good based on the analysis of AUCs (sensitivity and specificity) to better understand whether these indexes would be predictive in the recognition of dogs at risk of death. Linear indexes (SDNN, RMSSD, PNN50%) were more sensitive (95.45%) with a low rate of false negatives compared to other indexes in the identification of dogs at risk of death and had a higher negative predictive value (96.67%); dogs with SDNN, RMSSD and PNN50% higher than 34.7 ms, 19.0 ms and 2.54% (cut off), respectively, were less likely to die. On the other hand, the nonlinear indexes (REC%, DET% and LAM%) presented a higher specificity (100%) with a low rate of false positives compared to all other indexes in the identification of dogs at risk of death and had a higher positive predictive value (100%); dogs with REC, DET and LAM higher than 39.12%, 87.75% and 87.34%, respectively, had a higher risk of dying. Of all the indexes investigated, the ones with the highest diagnostic accuracy were RMSSD (99.8%), PNN50% (99.5%), SDNN (99.4%), ShanEnt (98.4%) and DET% (96.4%), which were clinically relevant, serving as useful tools to help determine the risk of death of a patient.

This is the first time in veterinary medicine that cutoff values for dogs at risk of death from HRV indexes have been evaluated, demonstrating the efficacy of HRV analysis as an accessible, low-cost and rapid research tool to evaluate the autonomic homeostasis of an individual independent of the existing disease, aiding in the prognosis of patients.

The limitations of this study were that the recording of RR intervals for HRV analysis was not standardized, so some patients may have been slightly more anxious, but the intention of using the heart rate monitor to perform the analysis was to generate the lowest level of stress in these dogs. All dogs at the time of analysis were apparently calm. Another limitation was the mean age of the animals in the control group, as they were very young dogs compared to the other groups. Although the number of patients in the chronic patient and death groups may be more robust, recurrence rates and linear indexes have proven to be useful tools in determining the different health statuses of dogs. These analyses served as predictors, thus enabling their use in veterinary clinical practice. A study with a larger number of patients with chronic morbid conditions is necessary.

CONCLUSION

The linear and non-linear indexes of HRV proved to be useful predictors in the assessment of homeostasis between dogs with morbid conditions (chronic diseases) and at risk of death and among healthy and life-threatening conditions in dogs. Linear indexes and the nonlinear DET% index were relevant to aid in the differentiation of healthy dogs from sick dogs. The qualitative analysis of recurrence plots showed usefulness in the rapid identification of health status in dogs. The present study allowed the identification of linear (RMSSD, PNN50%, SDNN) and nonlinear (ShanEnt, DET%) indexes, which presented greater diagnostic accuracy in the identification of patients at risk of death and predictive cutoff values; these results are important, as they may direct the veterinarian clinician to optimize the therapy instituted, aiming to improve the general condition of the patient or reinforce the evidence of clinical evolution, indicating whether the prognosis is good, reserved or poor.

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