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DELay of Appearance of sYmptoms of Canine Degenerative Mitral Valve Disease Treated with Spironolactone and Benazepril: the DELAY Study

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KEYWORDS
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Abstract *Introduction:* Efficacy of renin-angiotensin-aldosterone system (RAAS) blockade using angiotensin-converting enzyme inhibitors (ACEi) in dogs with preclinical myxomatous mitral valve disease (MMVD) is controversial.
Hypothesis: Administration of spironolactone (2—4 mg q 24 h) and benazepril (0.25—0.5 mg q 24 h) in dogs with preclinical MMVD, not receiving any other cardiac medications, delays the onset of heart failure (HF) and cardiac-related death. Moreover, it reduces the progression of the disease as indicated by echocardiographic parameters and level of cardiac biomarkers N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI).
Animals: 184 dogs with pre-clinical MMVD and left atrium-to-aortic root ratio (LA:Ao) $\geq$1.6 and normalized left ventricular end-diastolic diameter (LVEDDn) $\geq$1.7.
Methods: This is a prospective, randomized, multicenter, single-blinded, placebo-controlled study. Primary outcome variable was time-to-onset of first occurrence of HF or cardiac death. Secondary end points included effect of treatment on progression of the disease based on echocardiographic and radiographic parameters, as well as variations of NT-proBNP and cTnI concentrations.
Results: The median time to primary end point was 902 days (95% confidence interval (CI) 682-not available) for the treatment group and 1139 days (95% CI 732-NA) for the control group ($p = 0.45$). Vertebral heart score ($p = 0.05$), LA:Ao ($p < 0.001$), LVEDDn ($p < 0.001$), trans-mitral E peak velocity ($p = 0.011$), and NT-proBNP ($p = 0.037$) were lower at the end of study in the treatment group.
Conclusions: This study failed in demonstrating that combined administration of spironolactone and benazepril delays onset of HF in dogs with preclinical MMVD. However, such treatment induces beneficial effects on cardiac remodeling and these results could be of clinical relevance.

Abbreviations

ABT	Aldosterone breakthrough
ACEi	Angiotensin-converting enzyme inhibitor
CI	Confidence interval
cTnI	Cardiac troponin I
DELAY	DELay of Appearance of sYmptoms of canine degenerative mitral valve disease treated with spironolactone and benazepril
E peak	Early transmitral peak velocity
HF	Heart failure
ITT	Intention-to-treat population
LA:Ao	Left atrium-to-aortic root ratio
LVEDDn	Normalized left ventricular end-diastolic diameter
MMVD	Myxomatous mitral valve disease
NA	Not available
NT-proBNP	N-terminal pro brain natriuretic peptide
PP	Per protocol population
RAAS	Renin-angiotensin-aldosterone system
SCDS	Scientific committee for the DELAY study
U A:C	Urine aldosterone-to-creatinine ratio

Introduction

Myxomatous mitral valve disease (MMVD) is the most common cardiovascular disease observed in dogs and the most frequent cause of heart failure (HF) in this species [1,2]. The disease has a relatively long preclinical period defined as stage B1, characterized by the absence of radiographic or echocardiographic evidence of cardiac remodeling, and stage B2, when affected dogs present with evidence of left atrial and/or left ventricular enlargement [3,4]. Over the years, various medical interventions to delay the onset of HF in asymptomatic dogs affected by MMVD and cardiac remodeling have been evaluated by prospective, placebo-controlled, double-blinded multicenter clinical trials. In a large study involving 360 dogs with MMVD stage B2, the administration of pimobendan, a calcium sensitizer and phosphodiesterase-3 inhibitor, resulted in prolongation of the preclinical stage by approximately 15 months [5]. Conversely, two studies evaluating the effectiveness of enalapril, an angiotensin-converting enzyme inhibitor (ACEi), have failed to provide clear evidence of delaying the onset of HF, despite the clinical efficacy demonstrated in dogs with HF [6,7]. Such therapeutic failure could be attributed, at least in part, to an inefficient suppression of aldosterone secretion through a mechanism known as aldosterone breakthrough (ABT), especially when an ACEi is given for a relatively long time, with circulating aldosterone concentrations increasing above pretreatment levels [8]. Indeed, high levels of circulating aldosterone can contribute to the progression of heart disease in humans by promoting cardiac fibrosis and hypertrophy, as well as enhancing inflammatory cell function [9—11]. It appears that ABT is a common occurrence also in approximately 30% of dogs with preclinical MMVD receiving an ACEi [12]. Therefore, the addition of the mineralocorticoid receptor antagonist spironolactone to an ACEi could potentially delay the progression of heart disease in dogs with preclinical MMVD, by providing a more effective suppression of the renin-angiotensin-aldosterone system (RAAS). Based on these considerations, we hypothesized that the combined chronic administration of spironolactone and benazepril in dogs affected by MMVD with cardiac remodeling (stage B2), not receiving any other cardiac medications, can delay the onset of HF compared with similar dogs receiving placebo. We also hypothesized that chronic treatment with spironolactone and benazepril reduces the progression of the disease as indicated by specific imaging variables and level of cardiac biomarkers N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI).

Animals, material, and methods

Study design

The "DELay of Appearance of sYmptoms of canine degenerative mitral valve disease treated with spironolactone and benazepril" (DELAY) study was a prospective, multicenter, single-blinded, randomized, placebo-controlled study. The study protocol was designed by an independent group of cardiologists (M.B., C.B., D.C., F.M., L.F., G.D., M.P., R.A.S., the Scientific Committee for the DELAY study [SCDS]) in conjunction with the sponsor. The study was approved by the Italian, Dutch, and United Kingdom agencies for veterinary drugs. The study was approved by an ethical review committee at each site where this was required. The contract between the sponsor and the lead investigator (M.B.) stipulated that the latter would have full access to all results and the right to independently publish the study regardless of the outcome.

Dogs

Privately owned dogs were enrolled in twenty-one centers in Europe (sixteen in Italy, one in the Netherlands, and four in the United Kingdom) between November 2010 and June 2014 (Italy), December 2013 and August 2014 (the Netherlands), and April 2014 and September 2014 (the United Kingdom). The follow-up phase was terminated on 31 January 2018 in Italy, 30 September 2017 in the Netherlands, and 31 March 2018 in the United Kingdom. All authors but four (M.B., K.L., E.G., and C.G.P.) were also investigators recruiting cases.

The enrollment criteria were as follows: Dogs were eligible for participation in the study only if the owner had given informed written consent. To be eligible for inclusion, a dog had to be between 4 and 14 years of age, have a body weight $\geq$2.5 kg and $\leq$20 kg, have a left apical systolic murmur, and had to be in American College of Veterinary Internal Medicine (ACVIM), stage B2 of MMVD [3]. For this study, a B2 dog was defined by the presence of echocardiographic evidence of MMVD with cardiac remodeling, absence of current or previously reported clinical signs attributable to HF, and absence of pulmonary edema on thoracic radiographs. The diagnosis of MMVD was based on the presence of mitral valve leaflets thickening and/or mitral valve prolapse and the presence of mitral regurgitation on color Doppler study. In this study, cardiac remodeling was defined by a left atrium-to-aortic root ratio (LA:Ao) $\geq$ 1.6 [13] and a normalized left ventricular end-diastolic diameter (LVEDDn) $\geq$ 1.7 [14].

The exclusion criteria were as follows: Dogs were excluded from the study if they had cardiovascular disease other than MMVD (including congenital diseases), presence of atrial fibrillation documented by electrocardiographic recording, past treatment with cardiovascular drugs lasting more than two weeks or during the last two weeks before inclusion, systemic hypertension (defined as a systolic pressure over 180 mmHg on five repeated measurements), pulmonary hypertension (diagnosed by the presence of a tricuspid regurgitation velocity $>$ 3 m/s assessed by spectral Doppler study), and clinically relevant systemic disease.

Randomization and allocation

Dogs were allocated to treatment groups by randomization. A statistician was responsible for randomizing treatments for dogs enrolled in the study.[ac] A computer-generated randomization[ad] table was provided by the statistician to each clinic. Dogs were randomized based on the order of presentation at each site. At the time of enrollment, each dog was given a unique identification number and was assigned to a treatment code based on the randomization table for that site. Each investigator was assigned 24 consecutive study numbers. The maximum number of cases enrolled at any single center was 14.

Blinding

Each clinical investigator was blinded to the treatment codes and treatment assignment of subjects. Treatment was provided by an appointed dispenser who was aware of the treatment codes but kept this information strictly confidential and did not participate in or influenced the clinical evaluations. Although the study was designed to be single-blinded, interventions to maintain the owner blinded of treatment were attempted. These included the modification of the original packaging of the treatment drugs with dedicated blister wallets. Forms and other documents related to treatment identity were stored in a secure location at each site to maintain blinding. Owners and site personnel were given guidance to prevent them from disclosing accidentally information that would unblind the investigator. Drugs were dispensed in sealed cardboard boxes to prevent inadvertent disclosure of the treatment group of investigators.

Treatment groups

Spironolactone (Prilactone® 10 mg and 40 mg tablets) and benazepril chlorhydrate (Fortekor® 2.5 and 5 mg tablets) were administered PO at the target dose of 2—4 mg/kg q 24 h for spironolactone and 0.25—0.5 mg/kg q 24 h for benazepril. The calculated dose was adjusted to a suitable number of tablets. Placebo (10 mg and 40 mg tablets) was administered PO q 24 h, and all dogs received two placebo tablets q 24 h to match the treatment administration. Assessment of compliance was ensured by asking the owner to return empty, partially used, or unused blisters of tablets every three months. At each visit, the dispenser counted the remaining tablets and provided the correct number of tablets needed for three months. Lack of administration, noticed by the dispenser or reported by the owner at each visit for more than

[ac] Ceva Santé Animale Biostatistic Department.

[ad] PLAN procedure in SAS, SAS Institute, Cary NC.

ten consecutive days or more than 20% of the follow-up duration in total, was considered as a major deviation leading to removal from the analysis of data in the per protocol (PP) population.

Population analyzed

All dogs that were randomized and received at least one dose of the treatment or the placebo were included in the intention-to-treat (ITT) population. Only dogs for which the protocol was strictly respected could be included in the PP population. Dogs remained in the study until one of the following occurred: the dog reached the primary end point, the dog was censored from the primary end point analysis owing to occurrence of an event that precluded continuation of the study or the eighth follow-up visit was completed.

Concomitant treatment

Any concomitant treatment was under the responsibility of the investigator and had to be described in detail (name, concentration, daily dose, route of administration, justification) on the case report form. Forbidden concomitant treatments included any cardiovascular treatment other than the study drugs or any treatment that could interact with the evaluation of the tested product. In case of administration of any forbidden treatment, the dog was censored from the PP analysis. An exception for forbidden drug was the short-term administration of any cardiovascular drug for a period of less than seven days. Other concomitant medications, therapies or vaccines were allowed as long as they were considered not to be interacting with the evaluation of the tested product after approval by the lead investigator (M.B.). The use of such concomitant treatment had to be reserved only to guarantee the best standard of care of enrolled dogs.

Visit schedule

Eight mandatory visits were scheduled from day 0 to month 42 every 6 months. Additional visits were allowed for medical concerns. Table 1 summarizes the scheduled visits and diagnostics tests performed at each visit.

Clinical evaluation

At the inclusion, dogs' characteristics (breed, age, sex, body weight, and body condition score) were recorded. Clinical history and physical findings were documented at each visit.

Thoracic radiographs

Thoracic radiographs were performed at each scheduled visit. Cardiac size was assessed by the vertebral heart scale method [15]. Radiographs were first evaluated by the investigator and, in case of detection of pulmonary edema, this had to be confirmed by the lead investigator (M.B.) within 7 days. In case of discrepancy between the two evaluators (investigator and M.B.), a third opinion from a certified boarded radiologist, blinded to dog clinical status, had to be requested.

Table 1 Scheduled visit for each dog and diagnostic tests performed at each visit.

Visit No.	V1	V2	V3	V4	V5	V6	V7	V8[a]
Day/month	D0	M6	M12	M18	M24	M30	M36	M42
Physical examination	√	√	√	√	√	√	√	√
Serum CRT	√							
ECG	√							
Thoracic radiography	√	√	√	√	√	√	√	√
Echocardiography	√		√		√		√	√
Systolic blood pressure	√	√	√	√	√	√	√	√
NT-proBNP	√	√	√	√	√	√	√	√
cTnI	√	√	√	√	√	√	√	√
U A:C	√	√	√	√	√	√	√	√

cTnI, cardiac troponin I; NT-proBNP, N-terminal pro brain natriuretic peptide; Serum CRT, serum creatinine; U A:C, urinary aldosterone-to-creatinine ratio; ECG, electrocardiogram.

[a] or early termination visit.

Echocardiography

An echocardiographic examination was mandatory for visit 1, 3, 5, 7, and 8. The investigators had the option to perform an additional examination at the other visits. Echocardiographic examinations were performed on unsedated dogs as per the established standard for veterinary cardiology [16]. All measurements were taken from at least three consecutive cardiac cycles, and the mean was recorded. The following measurements were taken from the right parasternal short-axis view: LA:Ao obtained in two-dimensional view as described by Hansson et al. [13], and left ventricular diameter measured in M-mode from the short axis with the leading edge to inner edge method at the level of the papillary muscles. Left ventricular normalized dimensions were calculated as described [14]. Early (E peak) and late A transmitral inflow velocities were assessed by spectral Doppler from the left four chamber apical view.

Urinary aldosterone-to-creatinine ratio

Approximately 6 mL of urine sample were collected by free catch, catheterization, or cystocentesis at each visit from each dog, divided in four aliquots and immediately frozen at −20 °C. Samples were shipped frozen once a year to a central laboratory[ae] for determination of urine aldosterone-to-creatinine ratio (U A:C). The laboratory was blinded to the treatment group. Samples were maintained at −80 °C until the measurement of urine aldosterone, which was performed using a validated assay for canine species,[af] in accordance to the procedure indicated in the data sheet. Creatinine was determined using commercial biochemical assay.

NT-proBNP and cTnI

Approximately, 5 mL of venous blood were obtained from each dog at each visit. Until December 31, 2016, blood was collected into tubes containing ethylenediaminetetraacetic acid and specialized protease inhibitor to reduce the risk of sample degradation. Blood samples collected after January 1, 2017 were collected into tubes containing only ethylenediaminetetraacetic acid. Samples were centrifuged within 60 min after collection, plasma was separated, divided in two aliquots, and frozen before overnight shipment to a central laboratory.[ag] Samples were then divided into several aliquots and were frozen at −80 °C. Measurement of NT-proBNP was performed in batches with Idexx first-generation immunoassay[ah] for samples collected before December 31, 2016 and with a second-generation immunoassay method[ai] for sample processed after January 1, 2017. The equivalence between the two methods has been previously reported [17].

Samples for cTnI were frozen and shipped overnight, once a year, to a central laboratory[aj] and were stored at −80 °C for batched analysis. Concentration of cTnI was analyzed using an ELISA[ak] assay in accordance with the manufacturer's instructions as previously described [18].

All plasma analysis for NT-proBNP and cTnI were carried out in duplicate and assays performance was previously reported [18,19].

Quality of life

Quality of life was assessed by using a scoring system based on the following parameters: body weight, appetite, cough, exercise intolerance, and syncope (Table 2).

Primary end point

The primary end point was the time to cardiac death or onset of first occurrence of HF defined by the presence of either dyspnea and/or tachypnea (≥36 breaths/min at rest) that could not be explained by another disease based on clinical judgment by the investigator [20]. Radiographs were performed at the last visit if the dog was presented at the investigator clinic and if its health condition allowed it. They were used as a basis to either confirm pulmonary edema or to exclude any other reason that could explain the observed clinical signs. In case where it was not possible to perform radiographs, owing to critical

[ae] Department of Veterinary Sciences, University of Torino, Grugliasco, Italy.
[af] Cayman Chemical Aldosterone ELISA Kit, Cayman Chemicals, Ann Arbor, MI, USA.
[ag] IDEXX BioResearch, Vet Med Labor GmbH, Ludwigsburg, Germany.
[ah] Canine Cardiopet® proBNP test kit, IDEXX Laboratories Inc., Westbrook, ME.
[ai] Canine Cardiopet® proBNP test kit, IDEXX Laboratories Inc., Westbrook, ME.
[aj] Laboratory for clinical chemistry and transfusion medicine, Southern Älvsborg Hospital, Alingsås, Sweden.
[ak] Access Systems AccuTnI Assay, Beckman Coulter, Inc, Fullerton, CA.

Table 2 Baseline characteristics of the two treatment groups in the intention-to-treat population and their comparison. Continuous variables are reported as mean and standard deviation (SD) when normally distributed and as median and 75th and 25th percentile range if not. Categorical variables are reported as number (%).

	Variable	Treatment groups		P-Value
		Spironolactone + Benazepril n = 87	Placebo n = 92	
Dogs' characteristics	Age (years)	9.3 (2.1)	9.2 (2.1)	0.694
	Sex (M/F) (%)	62/25 (71/29)	56/36 (61/39)	0.143
	Breed (Mixed-breed/CKCS/Poodle/Dachshund/ Chihuahua/Jack Russell terrier/ Bichon Frise/Other) (%)	31/20/5/6/4/1/3/17 (36/23/6/7/5/1/3/19)	41/14/7/6/3/4/2/15 (45/15/8/7/3/4/2/16)	—
	Mixed breed vs other breeds	31/56 (36/64)	41/51 (45/55)	0.223
	CKCS vs other breeds	20/67	14/78	0.185
Dose of test medication	Daily dose medication (spironolactone/benazepril; mg/kg)	2.8 ± 0.6/0.3 ± 0.1	NA	—
Quality of life and respiratory variables	Appetite (normal (1), increased (2), decreased (3), anorexia (4)) (%)	84/3/0/0 (97/3/0/0)	91/1/0/0 (99/1/0/0)	0.285
	Cough (none (0)/occasional (1)/frequent (2)/persistent (3)) (%)	72/15/0/0 (83/17/0/0)	72/20/0/0 (78/22/0/0)	0.448
	Exercise intolerance (No (0)/Yes (1)) (%)	85/2 (98/2)	91/1 (99/1)	0.528
	Syncope (none (0)/≤4 per month (1)/>4 per month (2)) (%)	86/1/0 (99/1/0)	90/2/0 (98/2/0)	0.594
Physical examination variable	Body weight (kg)	10.0 ± 4.2	9.8 ± 4.2	0.770
	Body condition score (too thin (1−3), ideal (4−5), too heavy (6−9)) (%)	3/63/21 (4/72/24)	4/62/24 (4/69/27)	0.806
	Heart rate (beats/min)	117 (20)	121 (20)	0.290
	Femoral pulse (normal (1)/ weak (2)/pulse deficit (3)) (%)	86/1/0 (99/1/0)	91/1/0 (99/1/0)	0.968
	Dyspnea (no (0)/on effort (1)/at rest (2) (%)	87/0/0 (100/0/0)	92/0/0 (100/0/0)	—
	Tachypnea (No (0)/Yes (1)) (%)	85/2 (98/2)	89/3 (97/3)	0.696
	Respiratory sinus arrhythmia (No (0)/Yes (1)) (%)	40/47 (46/54)	41/51 (45/55)	0.850
	Systolic blood pressure (mmHg)	143 ± 17	143 ± 16	0.945
	Heart murmur intensity (mild (1−2)/ moderate (3−4)/severe (5−6)) (%)	2/76/7 (3/89/8)	4/76/12 (4/83/13)	0.207
Diagnostic imaging variables	VHS	11.2 ± 0.9	11.2 ± 0.8	0.921
	LVEDDn	1.9 ± 0.2	1.9 ± 0.2	0.740
	LVESDn	1.0 ± 0.2	1.1 ± 0.2	0.349
	LA:Ao	1.9 ± 0.2	1.9 ± 0.2	0.564
	E peak (m/s)	1.0 ± 0.2	1.0 ± 0.3	0.462
	A peak (m/s)	0.8 ± 0.2	0.8 ± 0.2	0.617
Laboratory variables	Urinary creatinine (mg/dL)	170.7 ± 109.3	182.2 ± 127.8	0.542
	Urinary aldosterone (pg/mL)	642.6 ± 473.8	657.3 ± 483.3	0.845
	U A:C	0.4 ± 0.5	0.4 ± 0.5	0.902
	NT-proBNP (pmol/L)	1150 (625−1718)	880 (632−1731)	0.277
	HS cTnI (ng/mL)	0.04 ± 0.10	0.04 ± 0.05	0.739

A peak, late transmitral peak velocity; E peak, early transmitral peak velocity; LVEDDn, normalized left ventricular end-diastolic diameter; LVESDn, normalized left ventricular end-systolic diameter; LA:Ao, left atrium-to-aortic root ratio; VHS, vertebral heart scale; U A:C, urinary aldosterone-to-creatinine ratio; NT-proBNP, N-terminal pro brain natriuretic peptide.

health conditions or refusal of the owner to run this examination, or if the dog was not directly seen by the investigator because of a sudden clinical deterioration (death at home, visit to their general practitioner), diagnosis of HF was determined by the investigator based on clinical signs observed, and/or information reported by the owner or by other veterinarians.

At the end of the study, an independent committee composed of three board-certified cardiologists[al], blinded to the treatment group and not involved in the study, reviewed all reasons for ending the study, and confirmed whether or not each individual reached the primary end point. All clinical data and radiographs were provided to this committee. In case of discrepancy between experts in the independent committee, the majority opinion was considered as the final decision (i.e. two experts vs one).

The independent committee provided the SCDS blinded data review committee (D.C., F.M., M.B., M.P.) with its final decisions. In case of discrepancy between the independent committee and the SCDS blinded data review committees, cases were discussed again and the opinion of the majority of all committees' members was considered as the final decision.

Secondary end points

The secondary end points of the study included evaluation of effect of treatment on progression of the disease based on echocardiographic and radiographic parameters, systolic blood pressure values, and variations of cardiac biomarkers (NT-proBNP and cTnI). Adverse events in the two groups were compared and analyzed, whereas a time-to-deterioration analysis was performed for quality of life parameters. The effects of treatment on urinary aldosterone and U A:C were also assessed.

Data management

All clinical observations and project activities were carefully recorded on the case report form. All study documentation, including the protocol, the raw data, and the statistical and clinical electronic reports, were stored at the sponsor's facility. Accuracy of the recorded data was checked on site by the appointed monitor. All data were entered into a database. After verification of the accuracy of data entries, a medical review was performed by the SCDS data review committee. After the database was locked, it was transferred to the independent statistician (K.L.). Blinding was maintained during data entry, audit, and statistical analysis.

[al] Sonja Fonfara, Dan Hogan and Matthias Schneider.

Statistical methods

The projected number of dogs required for the study was determined by the probability for an untreated dog to progress to HF during the study period. Based on previous published data, a dog has 60% of chance of not progressing into HF in a 3.5 years period [21]. Based on these data, a power analysis indicated that it was necessary to include 200 dogs to show significance with an alpha of 0.05 and a beta of 0.8 based upon a 22% difference in the time free of HF. Assuming a hypothetical 20% of withdrawal from the study, the inclusion of 240 animals was considered to represent a safe margin to identify any treatment effect in the 3.5 year follow-up period.

Baseline descriptive statistics are reported as mean and standard deviation for normally distributed variables and median and interquartile range for non-normally distributed variables. The distribution of residuals was assessed by visual inspection followed by the Kolmogorov-Smirnoff test. Between-groups analyses of baseline variables were performed using analysis of variance as error residuals were normally distributed. Interaction of categorical clinical covariates was carried out by analysis of variance, whereas interaction of categorical and continuous echocardiographic variables was carried out by analysis of covariance generating least square means for analysis. Analyses for proportions of categorical variables were evaluated with a Chi-squared test or Fisher's exact analysis as appropriate. Date of study entry was the time of clinical diagnosis, and data obtained at this time were evaluated in baseline analyses. Time-to-event analyses were carried out in univariate by way of Kaplan-Meier product limit estimates. Cox semiparametric regression models were used to generate univariate models. Covariates found significant were used in conjunction with treatment for multivariable models. The multivariable models were analyzed using interaction terms using enter and stepwise regression and enter method. Multicollinearity was assessed by variance inflation factor analysis and deemed acceptable. Covariates for the multivariate model were presented as both continuous and categorical. Continuous variables were categorized based on spline assessment, clinical rationale, and previous literature. Tests for proportionality were carried out by visual inspection of Schoenfeld residuals, negative log estimated survival distribution function, and formal hypothesis testing of covariate by log (time)

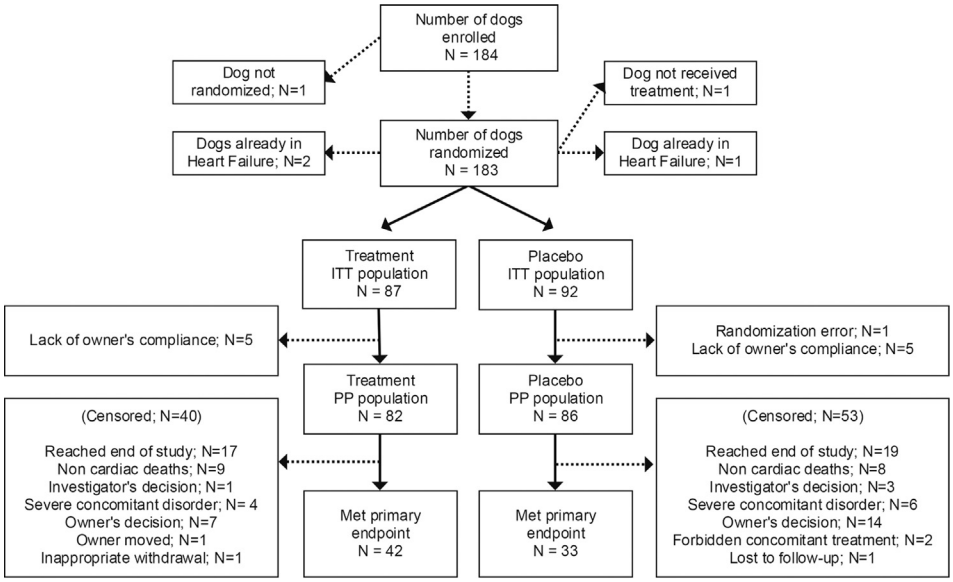

Fig. 1 Flow chart reporting the outcome for the 184 dogs randomized in the study.

interactions followed by Wald Chi-squared statistics and deemed proportional. Statistical differences between Kaplan-Meier product limit estimates strata were determined by a log-rank test. Time-to-event survival time analyses represented time from study entry to end date. Quality of life end points were defined as first date to clinical score deterioration or heart failure. Cases lost to follow-up or remaining alive were right censored. Analyses were performed with statistical software.[am] $P < 0.05$ was deemed significant.

During the course of the DELAY study, a related clinical trial reported the efficacy of pimobendan in a similar population of dogs [5]. For ethical reasons, an unplanned ad hoc interim analysis was carried out in the DELAY study. The interim analysis based upon a stopping probability at first look of 0.05 alpha suggested study continuation based upon the assumption that a required number of events were observed by the end of the predefined study follow-up period. After the interim analysis, owners of dogs still alive were informed about results of the EPIC trial [5] and were given the option of withdrawing their dog from the study to start administration of pimobendan. The final look of unadjusted *P* indicated an adjusted significance of 0.0475 alpha (0.95×0.05).

[am] SAS 9.4, 2016 Cary, NC: SAS Institute Inc.

Results

One hundred eighty-four dogs were enrolled and 183 randomized to receive trial treatment (Fig. 1). The median age of patients enrolled per investigator was 7.5 years (range, 5—13). Eighteen different breeds were included, with mixed breeds being the most represented (n = 72; 40%), followed by Cavalier King Charles Spaniels (n = 34; 19%), Poodles (n = 12; 7%), Dachshunds (n = 12; 7%), Chihuahuas (n = 7; 4%) Jack Russell terriers (n = 5; 3%), and Bichon Frise (n = 5; 3%). No differences were observed between the two groups at inclusion. In the treatment group, dogs received a mean daily dose of 2.8 ± 0.6 mg/kg of spironolactone and of 0.3 ± 0.1 mg/kg of benazepril. One dog was treated with pimobendan for less than two weeks before inclusion in the study. However, the treatment discontinued one month before the enrollment in agreement with the study design. A full summary of the ITT population characteristics is reported in Table 2.

In the PP population, the median duration in the study was 561 days (95% confidence interval [CI]: 142—977) and 75 dogs (44.9%) reached the composite primary end point (42 in the study group [51.2%] and 33 in the placebo group [38.4%], p = 0.46). Twenty-eight dogs (15.3%) died for cardiac causes (15 in the study group [20%] in the study group and 13 in the placebo group [17.3%], p = 0.88). The median time in the study of dogs reaching the primary end point was 1045 days [95% CI: 766-not available (NA)]. Ninety-three dogs

Table 3 Main reasons for censoring due to dog owner or investigator decision.

Owner decision	Spironolactone benazepril n = 7 No reason mentioned n = 2 Owner not motivated, did not want to continue, decided not to come back n = 3 Owner's personal problems n = 2	Placebo n = 14 No reason mentioned n = 3 Owner not motivated, did not want to continue, decided not to come back n = 3 Owner's personal problems n = 2 Owner concerns about study protocol: safety of study drugs or examinations n = 2 Owner wanted to start forbidden cardiac treatment n = 2 The owner decided to stop the study and suspicion of concomitant disorder n = 2
Investigator decision	Spironolactone benazepril n = 1 Investigator decision due to a suspected owner's lack of compliance n = 1	Placebo n = 3 Suspicion of concomitant disorder n = 1 Wrong diagnosis and prescription of a forbidden cardiac concomitant treatment n = 1 Investigator decision due to a suspected owner's lack of compliance n = 1

were censored from the analysis of end point (40 dogs in the treatment group and 53 in the placebo group), including 36 dogs that were still asymptomatic at the end of the study (17 in the treatment group and 19 in the placebo group). Twenty-five dogs (8 in the treatment and 17 in the placebo group) were censored from the study because of owner's or investigator's decision (Table 3).The other causes of censoring are reported in Fig. 1. The estimated median time to primary end point was 902 days (95% CI: 682-NA) for the treatment group and 1139 days (95% CI: 732-NA) for the control group (Fig. 2). There was no difference between groups in reaching the primary end point (p = 0.456). In the univariate Cox proportional hazard analysis, six variables were associated with

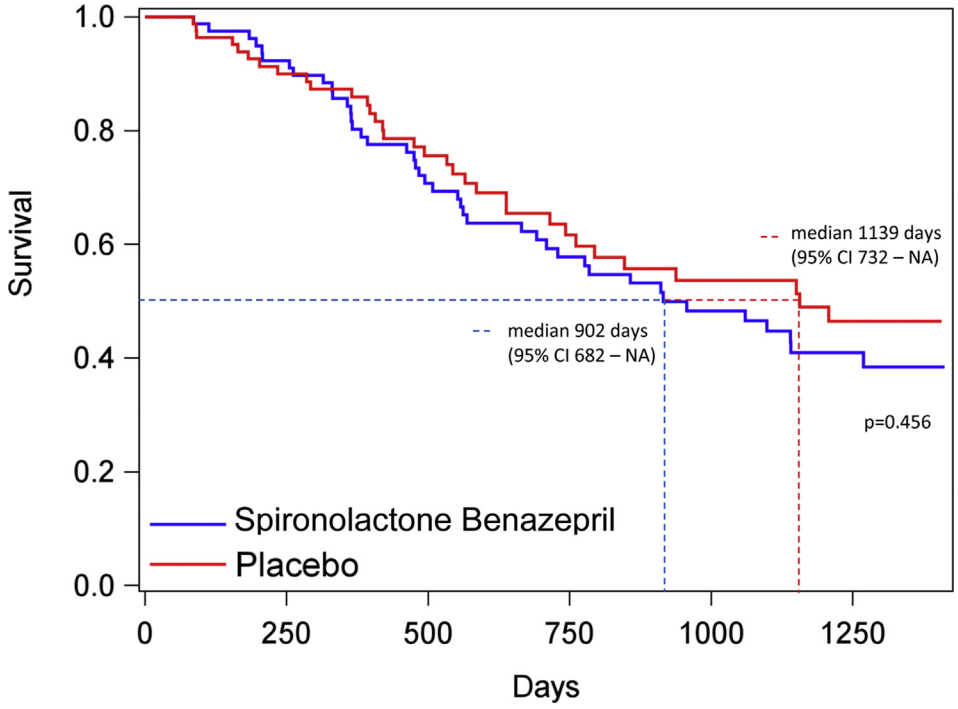

Fig. 2 Kaplan-Meier survival curves plotting the estimated percentage of dogs in each group in the per protocol population that have not reached the primary end point (heart failure or cardiac death) against time. There were 82 dogs in the benazepril spironolactone group and 86 dogs in the placebo group.

an increased risk for reaching the primary end point at $p < 0.05$ (Table 4). All these variables did not show any interaction and were not associated with treatment effects as demonstrated by the Cox multivariable analysis. Two of these variables, 10% LVEDDn increment (hazard ratio: 1.434, 95% CI: 1.264–1.626, $p < 0.001$) and 5 beats per minute heart rate increment (hazard ratio: 1.003, 95% CI: 1.001–1.005, $p = 0.012$), were independent predictors of an event in the Cox stepwise multivariate analysis.

An effect over the time of treatment with spironolactone and benazepril on several variables associated with cardiac remodeling was observed. The heart rate, LA:Ao, LVEDDn, and E peak velocity were lower at the end of study in the treatment group compared with the placebo group. The normalized left ventricular end-systolic diameter was smaller for V4 and V5 but was not different at the end of the follow-up. The vertebral heart scale was smaller for V5, V6, and V7 and nearing significant ($p = 0.053$) at the end of the study (Fig. 3, Table 5). The NT-proBNP, but not cTnI, was lower at the end of the study in the treatment group compared with the placebo group (Fig. 4, Table 6). The serum creatinine was not different among the groups at the time of enrollment in the study, and it did not change within and between groups over the time (Table 6). The urinary aldosterone concentrations were higher in the treatment group at the end of the study. The U A:C increased in both groups over the time (Fig. 4, Table 6).

In the ITT population, the median survival time was 925 days (95% CI: 789–1251). There were no differences in the number of adverse events between groups. There were 45 (44%) adverse events in the treatment group and 57 (56%) in the placebo group ($p = 0.235$). Table 7 provides detail of adverse events for each group. Concomitant treatments for each group are detailed in Table 8.

The estimated median time to primary end point was 902 days (95% CI: 656–1251) for the treatment group and 925 days (95% CI: 629-NA) for the placebo group (Fig. 5). Overall mortality for the ITT population was 31 of 179 dogs (17.4%), 15 (8.4%) in the spironolactone and benazepril group, and 16 (8.9%) for the placebo group.

There were no differences in quality of life parameters between the two groups.

Discussion

The DELAY study failed to demonstrate an effect of combined spironolactone-benazepril treatment in delaying the onset of HF in this population of asymptomatic dogs affected by MMVD despite successful blockade of the mineralocorticoid receptors, as demonstrated by the increased urinary aldosterone level, reduction or even reverse in cardiac remodeling on diagnostic imaging, and

Table 4 Probability to reach the primary end point in Cox proportional univariate analysis in the per protocol population. P-values <0.05 were considered significant.

	HR	95% CI	P value
Spironolactone/benazepril treatment	1.189	0.753–1.877	0.457
Body weight	1.015	0.961–1.072	0.603
Age	1.067	0.957–1.190	0.245
Heart rate (for 5 bpm increment)	1.002	1.000–1.005	0.040
SBP (for 10 mmHg increment)	1.001	0.999–1.002	0.430
CKCS (Yes)	1.226	0.661–2.274	0.518
VHS (for 0.1 increment)	1.380	1.059–1.798	0.017
LA:AO (for 10% increment)	1.129	1.030–1.237	0.010
LVEDDn (for 10% increment)	1.402	1.240–1.586	<0.001
LVESDn (for 10% increment)	0.958	0.838–1.094	0.522
E peak (for 10% increment)	1.101	1.010–1.200	0.028
A peak (for 10% increment)	1.087	0.972–1.215	0.145
NT-proBNP pmol/L (for 100 increment)	1.033	1.013–1.053	<0.001
cTnI ng/mL (for 10% increment)	0.913	0.625–1.334	0.639
U A pg/mL	1.000	0.999–1.000	0.083
U A:C	0.568	0.312–1.035	0.065

A peak, late transmitral peak velocity; bmp, beats per minute; CKCS, Cavalier King Charles spaniel; cTnI, cardiac troponin I; CI, confidence interval; E peak, early transmitral peak velocity; HR, hazard ratio; LVEDDn, normalized left ventricular end-diastolic diameter; LVESDn, normalized left ventricular end-systolic diameter; LA:Ao, left atrium-to-aortic root ratio; NT-proBNP, N-terminal pro natriuretic peptide; SBP, systolic blood pressure; U A, urine aldosterone; U A:C, urinary aldosterone-to-creatinine ratio.

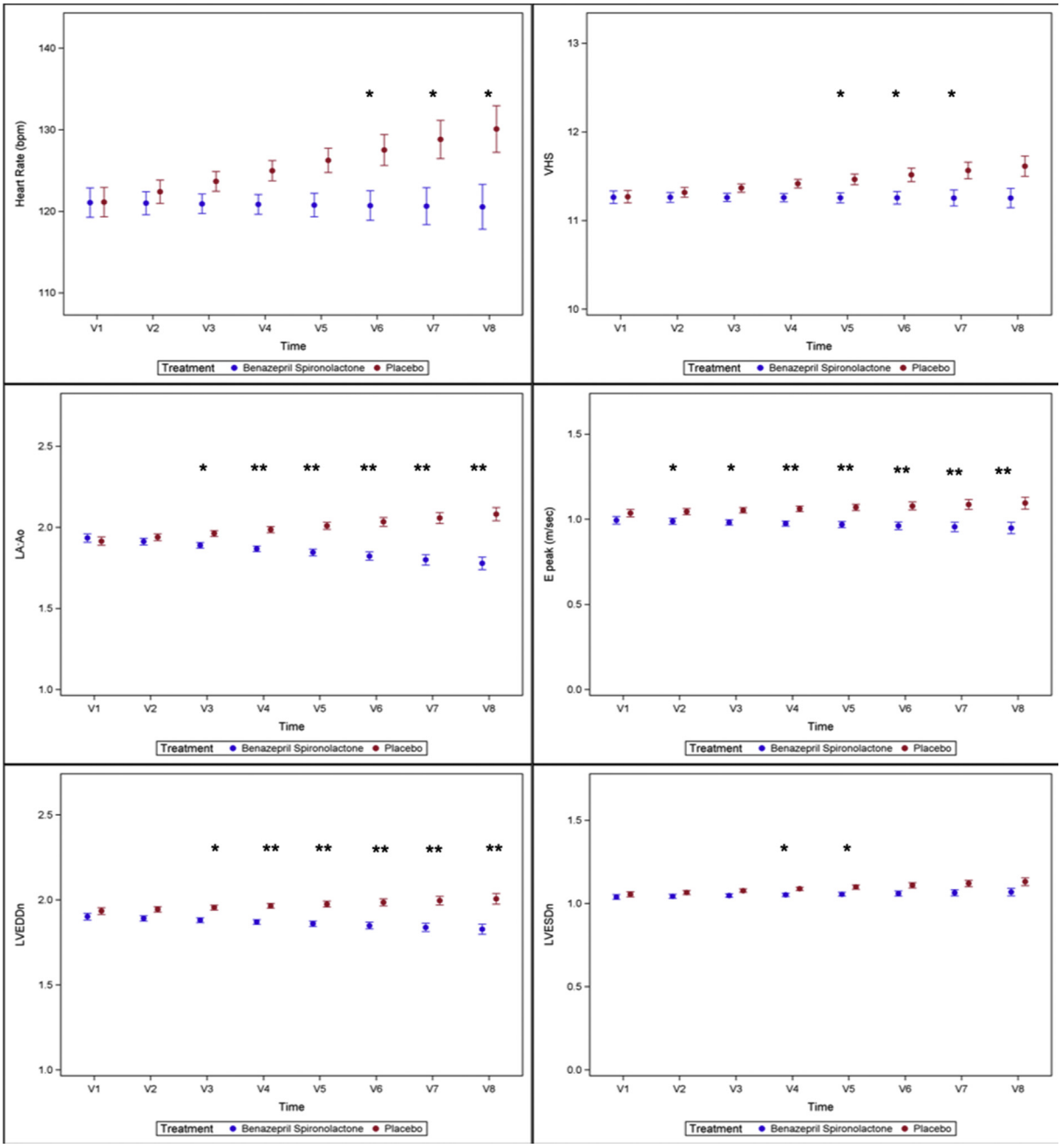

Fig. 3 Summary of analysis of covariance for heart rate and parameters of cardiac remodeling. There is a treatment effect over placebo for all variables (Table 4) over the time (*P < 0.05, **P < 0.001). BPM, beats per minute; E peak, early transmitral peak velocity; LA:Ao, left atrium-to-aortic root ratio; LVEDDn, normalized left ventricle end-diastolic diameter; LVESDn, normalized left ventricular end-systolic diameter; VHS, vertebral heart score.

lower concentrations of NT-proBNP in the treatment group at the end of the follow-up.

The lack of prolongation of the asymptomatic phase in the treatment group might be explained by several considerations. First, dogs with preclinical MMVD represent a heterogeneous group of animals with some individuals presenting a slow progression of the disease and other rapidly developing HF [4]. In an effort to enroll a more homogeneous group of dogs, the DELAY study had specific echocardiographic inclusion criteria, as had other previous studies [5,6]. However, these criteria still allow inclusion of dogs affected by a mild disease, as well as dogs with a more advanced condition. In our study, 35% of dogs had a LA:Ao < 1.8 and 27% a LVEDDn <1.8, suggesting that they were affected by a relative mild form of the disease and had therefore a lower risk of reaching the

Table 5 Summary of effects of treatment over time for radiographic and echocardiographic parameters. Values are reported as adjusted mean and standard error. The number of cases remaining in the study at each time point for the placebo and treatment were: V1, 81:86; V2, 73:69; V3, 59:60; V4, 47:44; V5, 36:34; V6, 33:26; V7, 24:24; and V8, 20:19. P-values <0.05 were considered significant.

Visit	Tx	HR bpm	P value	VHS	P value	LA:Ao	P value	LVEDDn	P value	LVESDn	P value	E peak (m/sec)	P value
V1	SB	121 ± 1.79	0.982	11.3 ± 0.07	0.899	1.92 ± 0.03	0.763	1.90 ± 0.02	0.440	1.04 ± 0.02	0.502	0.98 ± 0.02	0.123
	P	121 ± 1.79		11.3 ± 0.07		1.91 ± 0.03		1.92 ± 0.02		1.05 ± 0.02		1.03 ± 0.02	
V2	SB	121 ± 1.42	0.482	11.3 ± 0.06	0.685	1.90 ± 0.02	0.284	1.89 ± 0.02	0.066	1.04 ± 0.01	0.249	0.98 ± 0.02	0.020
	P	122 ± 1.42		11.3 ± 0.06		1.94 ± 0.02		1.93 ± 0.02		1.06 ± 0.01		1.04 ± 0.02	
V3	SB	121 ± 1.20	0.104	11.3 ± 0.05	0.251	1.88 ± 0.02	0.003	1.88 ± 0.01	0.002	1.05 ± 0.01	0.085	0.98 ± 0.02	0.001
	P	124 ± 1.21		11.4 ± 0.05		1.96 ± 0.02		1.94 ± 0.01		1.08 ± 0.01		1.06 ± 0.02	
V4	SB	121 ± 1.20	0.017	11.3 ± 0.05	0.074	1.87 ± 0.02	<0.001	1.87 ± 0.01	<0.001	1.06 ± 0.01	0.037	0.97 ± 0.02	<0.001
	P	125 ± 1.24		11.4 ± 0.05		1.99 ± 0.02		1.96 ± 0.01		1.09 ± 0.01		1.06 ± 0.02	
V5	SB	121 ± 1.44	0.009	11.3 ± 0.06	0.042	1.85 ± 0.02	<0.001	1.86 ± 0.02	<0.001	1.06 ± 0.01	0.037	0.97 ± 0.02	<0.001
	P	126 ± 1.49		11.5 ± 0.06		2.01 ± 0.02		1.97 ± 0.02		1.10 ± 0.01		1.06 ± 0.02	
V6	SB	121 ± 1.82	0.009	11.3 ± 0.07	0.040	1.83 ± 0.03	<0.001	1.85 ± 0.02	<0.001	1.07 ± 0.02	0.055	0.97 ± 0.02	0.002
	P	128 ± 1.89		11.5 ± 0.08		2.04 ± 0.03		1.98 ± 0.02		1.11 ± 0.02		1.07 ± 0.02	
V7	SB	121 ± 2.27	0.012	11.3 ± 0.09	0.046	1.81 ± 0.03	<0.001	1.84 ± 0.02	<0.001	1.07 ± 0.02	0.079	0.97 ± 0.03	0.005
	P	129 ± 2.35		11.5 ± 0.09		2.06 ± 0.03		1.99 ± 0.03		1.12 ± 0.02		1.08 ± 0.03	
V8	SB	121 ± 2.75	0.016	11.3 ± 0.11	0.054	1.79 ± 0.04	<0.001	1.84 ± 0.03	<0.001	1.08 ± 0.02	0.105	0.97 ± 0.03	0.011
	P	130 ± 2.85		11.6 ± 0.12		2.09 ± 0.04		2.00 ± 0.03		1.13 ± 0.02		1.09 ± 0.03	

Bpm, beats per minute; E peak, early transmitral peak velocity; HR, heart rate; LA:Ao, left atrium-to-aortic root ratio; LVEDDn, normalized left ventricular end-diastolic diameter; LVESDn, normalized left ventricular end-systolic diameter; P, placebo; SB, spironolactone benazepril; Tx, treatment; VHS, vertebral heart scale.

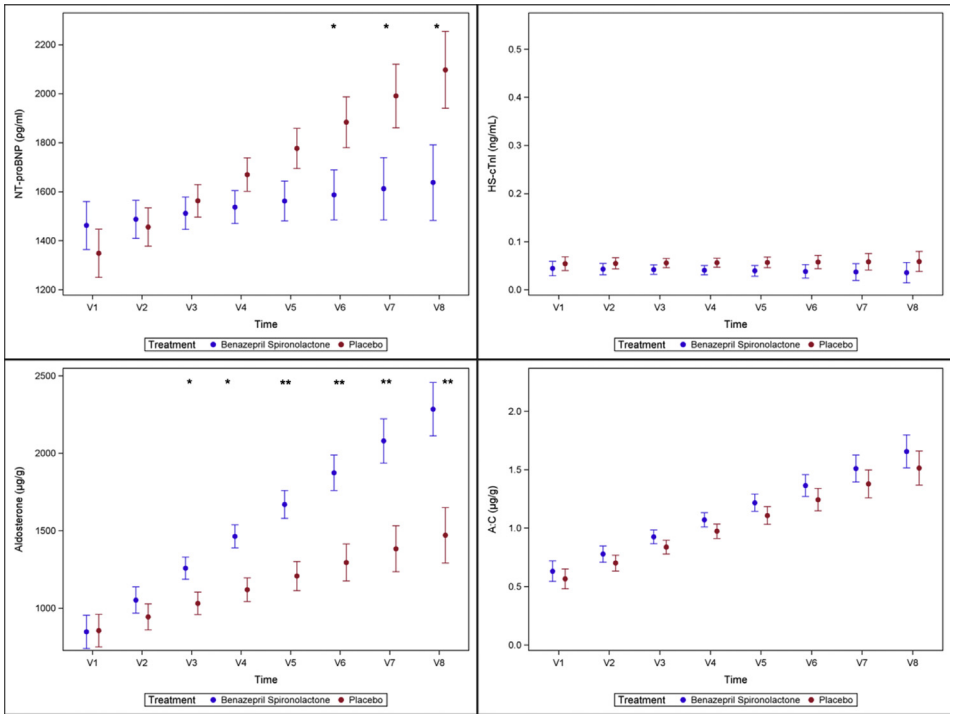

Fig. 4 Summary of analysis of covariance for cardiac biomarkers, urinary aldosterone and urinary aldosterone-to-creatinine ratio. There is a treatment effect over placebo (*P < 0.05, **P < 0.001) for N-terminal pro brain natriuretic peptide, and the urinary aldosterone is significantly higher in the treatment group over the time (Table 4). NT-proBNP, N-terminal pro natriuretic peptide; cTnI, cardiac troponin I; U A, urine aldosterone; U A:C: urinary aldosterone-to-creatinine ratio.

primary end point during follow-up. Studies testing efficacy of a treatment in a low-risk population normally require a higher number of patients or a longer observation period to demonstrate an effect on the natural history of the disease [22]. Pharmacological failure would be another potential explanation for this outcome. However, this seems unlikely because the increased urinary aldosterone in the treatment group indicates successful pharmacological blockade of mineralocorticoid receptors. Furthermore, the effects of treatment on echocardiographic and radiographic variables of cardiac remodeling and level of NT-proBNP would suggest an adequate pharmacological action on the RAAS. Finally, the DELAY study had a relatively small population of Cavalier King Charles spaniel compared with a similar trial [5]. Indeed, it has been reported that this breed has a faster progression from the preclinical to the clinical phase of the disease [23,24], therefore it is possible that this has influenced the rate of events in our study.

Despite failing to show a clinical benefit on the time to the onset of HF, the DELAY study suggests beneficial effects of the chronic treatment with combined spironolactone and benazepril. These include the slower progression or even improvement of echocardiographic and radiographic parameters in the treatment group. The vertebral heart scale, LA:Ao, LVEDDn, and E peak velocity of mitral valve inflow, which are all negative predictive parameters of outcome in previous studies [5,21,25,26], were significantly lower in the treatment group than the placebo group during the observation period. Furthermore, NT-proBNP concentration increases in the placebo group but not in dogs receiving treatment. The NT-proBNP is a biomarker that reflects myocardial stretch and has been associated with the severity of the disease and clinical outcome in previous studies [27—31]. All together, these results suggest that the combined spironolactone and benazepril treatment has a significant effect over time to reduce, or even reverse, cardiac remodeling. cTnI levels were not affected by treatment, confirming that there is not significant underlying myocardial damage during the preclinical phase of MMVD [18,32].

Results of this study suggest that the RAAS is activated in dogs with preclinical MMVD as the diseases progresses. This is demonstrated by the

Table 6 Summary of effects of treatment over time on cardiac biomarkers, urinary aldosterone, and urinary aldosterone-to-creatinine ratio. Values are reported as adjusted mean and standard error. P-values <0.05 were considered significant.

Visit	Tx	NT-proBNP pmol/L	P value	cTnI ng/mL	P value	Serum creat. mg/dL	P value	U A pg/mL	P value	U Creat. mg/dL	P value	U A:C μg/g	P value
V1	SB	1463 ± 98	0.415	0.04 ± 0.02	0.632	66.0 ± 1.9	0.979	822 ± 111	0.754	177.9 ± 8.6	0.680	0.60 ± 0.09	0.758
	P	1349 ± 98		0.04 ± 0.02		66.0 ± 2.0		872 ± 110		182.9 ± 8.5		0.56 ± 0.09	
V2	SB	1487 ± 78	0.776	0.03 ± 0.02	0.483	66.2 ± 1.5	0.753	1038 ± 88	0.517	172.3 ± 6.8	0.960	0.75 ± 0.07	0.560
	P	1456 ± 78		0.04 ± 0.02		66.8 ± 1.6		957 ± 87		172.8 ± 6.8		0.70 ± 0.07	
V3	SB	1512 ± 66	0.588	0.03 ± 0.02	0.335	66.3 ± 1.3	0.430	1253 ± 74	0.047	166.7 ± 5.8	0.622	0.91 ± 0.06	0.361
	P	1563 ± 66		0.13 ± 0.02		67.7 ± 1.3		1043 ± 75		162.6 ± 5.8		0.84 ± 0.06	
V4	SB	1537 ± 67	0.165	0.08 ± 0.03	0.263	66.4 ± 1.3	0.230	1469 ± 76	0.002	161.0 ± 5.9	0.311	1.07 ± 0.06	0.269
	P	1670 ± 68		0.04 ± 0.03		68.6 ± 1.3		1129 ± 78		152.5 ± 6.1		0.97 ± 0.06	
V5	SB	1562 ± 81	0.063	0.03 ± 0.03	0.285	66.7 ± 1.5	0.176	1684 ± 92	<0.001	155.4 ± 7.1	0.202	1.23 ± 0.07	0.275
	P	1777 ± 82		0.03 ± 0.03		69.5 ± 1.5		1214 ± 95		142.3 ± 7.4		1.11 ± 0.07	
V6	SB	1587 ± 102	0.042	0.03 ± 0.03	0.347	66.7 ± 1.9	0.178	1899 ± 117	<0.001	146.8 ± 8.9	0.176	1.38 ± 0.09	0.315
	P	1884 ± 104		0.04 ± 0.04		70.4 ± 1.9		1300 ± 121		132.2 ± 9.4		1.25 ± 0.09	
V7	SB	1612 ± 127	0.037	0.03 ± 0.04	0.410	66.8 ± 2.4	0.194	2115 ± 146	<0.001	144.1 ± 11.2	0.172	1.54 ± 0.11	0.356
	P	1991 ± 129		0.05 ± 0.03		71.2 ± 2.4		1386 ± 150		122.0 ± 11.7		1.39 ± 0.12	
V8	SB	1637 ± 154	0.037	0.03 ± 0.04	0.464	67.0 ± 2.9	0.211	2330 ± 178	<0.001	138.5 ± 13.6	0.175	1.70 ± 0.14	0.392
	P	2098 ± 157		0.06 ± 0.04		72.1 ± 2.9		1472 ± 181		111.9 ± 14.2		1.53 ± 0.14	

cTnI, cardiac troponin I; NT-proBNP, N-terminal pro natriuretic peptide; P, placebo; SB, spironolactone benazepril; Serum Creat, serum creatinine; Tx, treatment; U A, urine aldosterone; U A:C, urinary aldosterone-to-creatinine ratio; U Creat, urine creatinine.

Table 7 Adverse events and serious adverse events (adverse event which results in death, life-threatening, persistent disability) experienced by the dogs in the spironolactone + benazepril group and the placebo group (dogs could experience more than one adverse event or more than one clinical sign at the time of the adverse event).

	Spironolactone + benazepril		Placebo		P-value
	Adverse event	Serious adverse event	Adverse event	Serious adverse event	
Blood and lymphatic system disorders	1	0	1	0	0.866
Cardiovascular system disorders	5	1	5	3	0.919
Digestive tract disorders	6	1	10	0	0.789
Endocrine system disorders	2	0	1	0	0.425
Eye disorders	4	0	0	0	0.022
Hepatobiliary disorders	0	0	2	0	0.204
Musculoskeletal disorders	5	0	1	1	0.132
Neurological disorders	3	2	5	5	0.362
Renal and urinary disorders	3	0	2	3	0.695
Reproductive system disorders	1	0	1	0	0.866
Respiratory tract disorders	2	0	4	3	0.166
Skin and appendages disorders	2	0	3	0	0.849
Systemic disorders	4	1	6	0	0.925
Unclassifiable event	2	0	1	0	0.425
	40	5	42	15	
Total of events (serious and non-serious)	45		57		0.235
Number of dogs experiencing at least 1 event	13		21		0.179

increase of U A:C in the placebo group. The observed increased U A:C in the treatment group cannot be interpreted as evidence of ABT because of the effects on spironolactone on aldosterone metabolism. Aldosterone urine concentration is expected to be increased in patients receiving spironolactone as the result of mineralocorticoid receptor blockade. Ames et al. [12] have reported that U A:C ratio is significantly increased in dogs affected by MMVD with or without HF treated with spironolactone in addition to furosemide, pimobendan, and an ACEi compared with dogs treated with the standard triple therapy. Although the U A:C ratio does not differ between the group, urinary aldosterone concentration are significantly higher starting from visit two, suggesting a chronic pharmacological effect of the drug because the renal function was similar in both groups. The DELAY study also suggests that the pharmacological modulation of RAAS requires time to demonstrate its beneficial effects as indicated by the echocardiographic variable of cardiac remodeling and level of biomarkers that become different just after 12—18 months of treatment. Such results can be explained by the long time required by the RAAS pharmacological modulation to take effect, as reported in several human and veterinary clinical trials [33—35], as well as by the slow progression of the disease. Previous studies using an ACEi alone have not only failed to demonstrate a convincing effect on the time to the onset of HF in dogs with preclinical MMVD, but they also did not show any effect on cardiac remodeling assessed by diagnostic imaging [6,7]. It is possible that a more complete blockade of the RAAS by the combined treatment

Table 8 Concomitant treatments related to end of study, end point, or premature withdrawal.

		Spironolactone benazepril (n = 87)		Placebo (n = 92)	
Cardiac cause		Number of dogs N = 5		Number of dogs N = 2	
		Treatments prescribed (nb treatments)	Reasons for administration	Treatments prescribed (nb treatments)	Reasons for administration
	Cardiac treatments	Antiarrhythmic (2)	Atrial fibrillation	Inodilator (1)	Pulmonary hypertension
			Ventricular arrhythmia		
		ACEi (1) -MRA (1)	Pulmonary hypertension		
			Pulmonary veins congestion		
	Non-cardiac treatments	Cough suppressant (2)	Cough	Antiinfectives (1)	Diarrhea
				Gastroprotector (1)	Diarrhea
Non-cardiac cause		Number of dogs N = 4		Number of dogs N = 4	
		Treatments prescribed (nb treatments)	Reasons for administration	Treatments prescribed (nb treatments)	Reasons for administration
	Cardiac treatments		/		/
	Non-cardiac treatments	Antiinfectives (3)	Encephalitis	Antiinfectives (2)	Urinary tract infection
			Gastrointestinal syndrome		Tracheitis
		Anxiolytics (1)	Encephalitis	Anxiolytics (1)	Urinary tract obstruction
		Corticosteroids (2)	Lymphoma	Electrolytes (1)	Dehydration
			Encephalitis	Corticosteroids (4)	Neurological symptoms
		Insulin/ hormonotherapy (2)	Diabetes		Cough
			Hyperadrenocorticism		Paralysis
		Antiemetic (1)	Gastrointestinal syndrome		Pain
		Gastroprotector (1)	Gastrointestinal syndrome	NSAIDs (1)	Tracheitis
		Opioids (1)	Abdominal pain	Opioids (1)	Pain
				Gastroprotector (1)	Gastroprotection

ACEi, angiotensin-converting enzyme inhibitors.

with an ACEi and a mineralocorticoid receptor antagonist is able to effectively reduce its biological effects on cardiac remodeling [9], and the results of this study appear to confirm this hypothesis.

Limitations

The first limitation of this study was the insufficient number of cases of an advanced preclinical population enrolled, which affected the statistical power of the study as aforementioned. The second limitation was the unplanned prolonged recruitment period, which may have caused loss of motivation in recruiting cases, together with new findings becoming available. The third limitation is represented by the percentage of cases that reached the primary end point. In addition to the anticipated dropout ratio, some owners elected to abandon the study to administer pimobendan to their dogs after the publication of the clinical efficacy of administration of this drug in a comparable canine population. The length of the observational period may also appear excessively long for some owners, especially in apparently

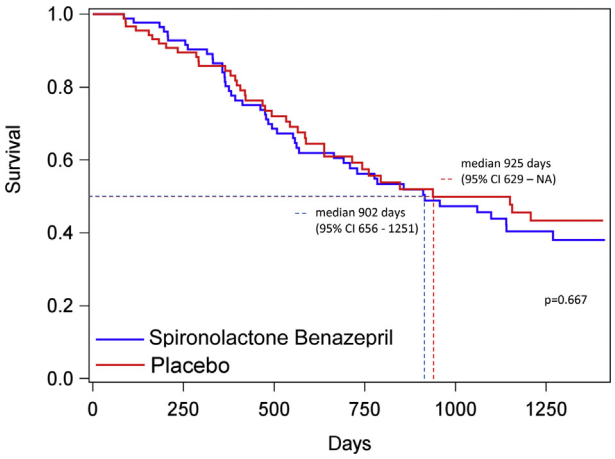

Fig. 5 Kaplan-Meier survival curves plotting the estimated percentage of dogs in each group in the intention-to-treat population that have not reached the primary end point (heart failure or cardiac death) against time. There were 87 dogs in the benazepril spironolactone group and 92 dogs in the placebo group.

healthy dogs, affecting their overall compliance. Finally, the presence of significant systemic disease was based on the assessment made by the investigators and this not always included a complete hemogram and serum biochemistry.

As disclosed in other studies [5,6], diagnosis of HF and confirmation of cardiac-related death represents a challenge. In particular, verification of HF based on thoracic radiographs is often controversial, despite independent blinded verification of the primary end point. For this reason, in this study, we used the term HF rather than congestive HF as primary end point. Moreover, not all diagnoses of the primary end point were supported by radiographic evidence of HF (e.g. sudden death, presentation to emergency clinics, etc). In such cases, a presumptive diagnosis of HF was based on clinical assessment and clinical history but this was equally observed in both groups and should not have affected the final results.

Conclusions

This study failed to demonstrate a prolongation of the asymptomatic phase of the disease in this population of dogs. Nevertheless, the treatment appears effective in reducing or even in reverse cardiac remodeling as indicated by various cardiac imaging parameters and NT-proBNP concentrations. Chronic administration of combined spironolactone and benazepril treatment in dogs with preclinical MMVD and cardiac enlargement is safe and well tolerated. The significant effect of this treatment on secondary end points could be of clinical relevance and merits further study.

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Conflicts of Interest Statement

Two authors, E.G. and C.G-.P., are representatives of Ceva Santé Animale. All the other authors have received funding from Ceva Santé Animale within the last 5 years for some or all of the following activities: research, travel, speaking fees, consultancy fees, and preparation of educational materials.

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