

Efficacy of Spironolactone on Survival in Dogs with Naturally Occurring Mitral Regurgitation Caused by Myxomatous Mitral Valve Disease

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Background: Spironolactone, an aldosterone antagonist, has been demonstrated to decrease mortality in human patients when added to other cardiac therapies.

Hypothesis: Spironolactone in addition to conventional therapy increases survival compared with conventional therapy in dogs with naturally occurring myxomatous mitral valve disease (MMVD).

Animals: Between February 2003 and March 2005, 221 dogs were recruited in Europe. Nine dogs were excluded from analysis, leaving 212 dogs with moderate to severe mitral regurgitation (MR) caused by MMVD (International Small Animal Cardiac Health Council classification classes II [n = 190] and III [n = 21]).

Methods: Double-blinded, field study conducted with dogs randomized to receive either spironolactone (2 mg/kg once a day) or placebo in addition to conventional therapy (angiotensin converting enzyme inhibitor, plus furosemide and digoxin if needed). Primary endpoint was a composite of cardiac-related death, euthanasia, or severe worsening of MR.

Results: Primary endpoint reached by 11/102 dogs (10.8%) in the spironolactone group (6 deaths, 5 worsening) versus 28/110 (25.5%) in control group (14 deaths, 8 euthanasia, 6 worsening). Risk of reaching the composite endpoint significantly decreased by 55% (hazard ratio [HR] = 0.45; 95% confidence limits [CL], 0.22–0.90; log rank test, $P = .017$). Risk of cardiac-related death or euthanasia significantly reduced by 69% (HR = 0.31; 95% CL, 0.13–0.76; $P = .0071$). Number of dogs not completing the study for cardiac and other miscellaneous reasons similar in spironolactone (67/102) and control groups (66/110).

Conclusion and Clinical Importance: Spironolactone added to conventional cardiac therapy decreases the risk of reaching the primary endpoint (ie, cardiac-related death, euthanasia, or severe worsening) in dogs with moderate to severe MR caused by MMVD.

Key words: Canine; Clinical trial; Evidence based medicine; Heart failure

The pathophysiology of congestive heart failure (CHF) in dogs involves several neurohormonal systems, such as the adrenergic system, the renin-angiotensin-aldosterone system (RAAS), and endothelin. All of these systems act to compensate for deteriorating cardiac function, but some have long-term deleterious effects and promote progression of CHF. Aldosterone is known to mediate the retention of water and sodium and consequently increases extracellular fluid volume, leading to increased cardiac preload.¹ Furthermore, aldosterone has a direct effect on the myocardium and vascular endothelium.^{2–4} Chronic exposure to high concentrations of aldosterone has been demonstrated in humans and rodents to promote fibrosis which is considered to be detrimental in cardiac disease.^{1,5,6} Angiotensin converting enzyme inhibitors (ACEIs) and furosemide

Abbreviations:

ACEI	angiotensin converting enzyme inhibitor
CHF	congestive heart failure
CL	confidence limits
DCM	dilated cardiomyopathy
GCP	Good Clinical Practice
HF	heart failure
HR	hazard ratio
ISACHC	International Small Animal Cardiac Health Council
MMVD	myxomatous mitral valve disease
MR	mitral regurgitation
RAAS	renin-angiotensin-aldosterone system
RALES	Randomized Aldactone Evaluation Study
VHS	vertebral heart size

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commonly are prescribed for the treatment of CHF. ACEIs suppress the RAAS cascade, but the extent of angiotensin converting enzyme (ACE) suppression appears insufficient to fully prevent aldosterone secretion in human or canine CHF patients receiving conventional therapy.^{7–10} In particular, diuretics, such as furosemide, have been shown to stimulate aldosterone secretion.¹¹ The addition of a selective aldosterone receptor antagonist therefore appears to be indicated in this setting. Spironolactone, a synthetic 17-lactone, is a competitive mineralocorticoid receptor antagonist.¹² In human patients suffering from CHF, the Randomized Aldactone Evaluation Study (RALES) demonstrated the beneficial effect of spironolactone when added to conventional therapy (ACEI, loop diuretic and digoxin).¹³ The finding

of a 31% reduction in risk of mortality (cardiac causes) in patients receiving spironolactone led to a premature termination of the study for ethical reasons. Because aldosterone is thought to be similarly involved in the pathophysiology of canine heart failure (HF), clinical field studies were conducted to test the hypothesis that dogs receiving spironolactone in addition to conventional therapy would have a favorable outcome compared with dogs receiving placebo plus conventional therapy. The aim of the trial was to assess the effect of spironolactone therapy on the risk of sudden cardiac death, euthanasia for cardiac reasons, or worsening of HF when compared with placebo in dogs with moderate to severe mitral regurgitation (MR) caused by myxomatous mitral valve disease (MMVD).

Materials and Methods

Animals

Two hundred and twenty-one dogs were enrolled from 32 practices in France (94), Germany (81), Belgium (42), and Italy (4), between February 2003 and March 2005. The study was completed in May 2006. Dogs consisted of 1st opinion and referred cases.

Study Design

This multicenter study was a prospective, double-blinded, placebo-controlled, randomized study. The complete clinical trial process (conception, monitoring, data management, analyses, and reporting) was conducted according to Good Clinical Practice (GCP)¹⁴ and European Medicines Agency requirements. Owners gave informed consent before beginning the study and had the option to withdraw their dog from the study at any time.

At the time of planning, most veterinary market authorization drug trials had been conducted over a few months time period. The

study was designed with a short-term evaluation and also a long-term evaluation to investigate the chronic effects of the tested drug. Thus, the follow-up was divided into 2 consecutive stages. In the 1st stage, dogs were recruited into 2 separate studies: one 2-month study, where furosemide treatment was mandatory at inclusion, and one 3-month study, where furosemide was not allowed at the time of inclusion. Over a short period of evaluation, the homogeneity of treatment was considered critical and justified the 2 separate protocols. Both studies were conducted in parallel by the same evaluation protocol and end-points. The 2nd stage was a 12-month study involving dogs that had completed either of the 1st studies (Fig 1). Dogs that completed the 1st studies were entered into the 12-month study, where they continued to receive the same trial treatment.

Inclusion Criteria

At initiation of the 1st stage, dogs of any breed or sex were enrolled when they presented with moderate to severe MR caused by MMVD (International Small Animal Cardiac Health Council classification [ISACHC] classes II and III). The original study protocol also included dogs with dilated cardiomyopathy (DCM), but these dogs were excluded from analyses because of a low number of included cases (see "Results"). Confirmation of MMVD as the underlying cardiac disease and disease severity was made by findings characteristic of MMVD, (ie, typical valve lesions and MR) at the echocardiographic examination¹⁵ and identification of heart enlargement on thoracic radiography (vertebral heart size [VHS] >10.5).¹⁶ To be included, dogs must furthermore have presented with at least 3 of the following clinical signs, including at least 1 of cough, dyspnea, syncope, and at least 1 of decreased activity, decreased mobility, altered demeanor.

All dogs were receiving an ACEI prescribed either at the time of or before inclusion in the 3-month study or at least 1 month before the 2-month study. Any ACEI with marketing authorization in Europe was permitted at the dosage recommended by the manufacturer. Furosemide therapy was prohibited at inclusion for the 3-month study and mandatory at inclusion for the 2-month study.

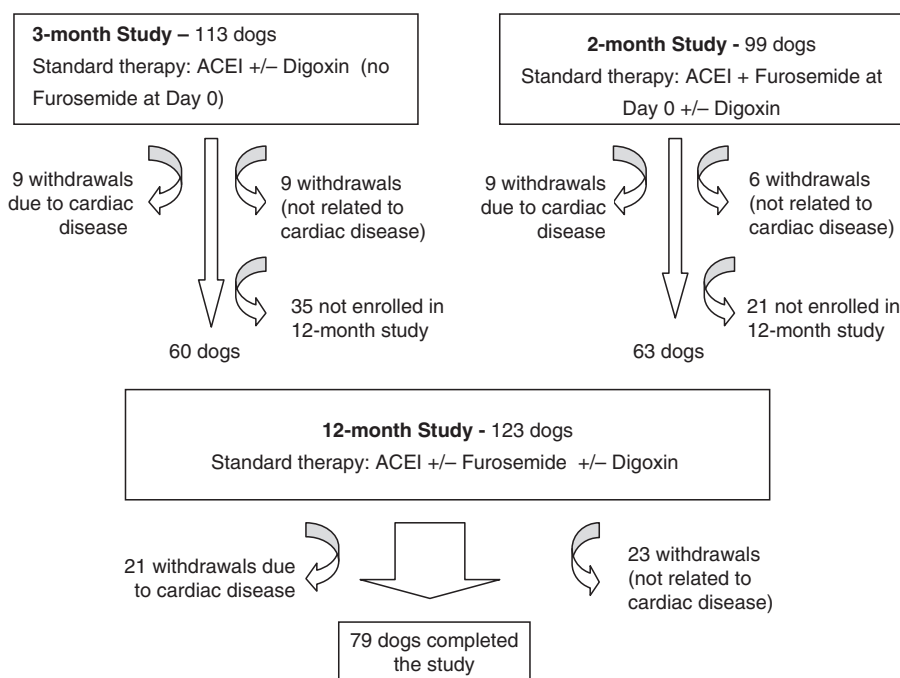


Fig 1. Design of the studies.

All dogs having completed 1 of the initial 2 studies could be enrolled in the 12-month study, if agreed by the owner.

Exclusion Criteria

Dogs were not enrolled if they were receiving cardiac medications other than ACEI, furosemide, digoxin, and L-carnitine. They were not included if they presented with acute pulmonary edema, a congenital cardiac disease, or a life-threatening arrhythmia, or if they presented with any other diagnosed medical condition (eg, diabetes mellitus, hyperadrenocorticism, neoplasia, severe renal, or hepatic disease), or if they were treated with any drugs that could interfere with the assessment of the tested product efficacy (eg, β -blockers, calcium channel inhibitors). Pregnant females were not enrolled because no safety data were available for these dogs.

Randomization and Blinding Conditions

Lists of randomization were set by computer software by blocks of 6, with a 1:1 ratio, to assure an equivalent number of dogs in each treatment group. Investigators received a set of treatment boxes bearing 6 consecutive numbers corresponding to a block. Each treatment box was identified by the randomization number. Because placebo was identical in appearance and packaging to spironolactone, neither the veterinarian nor the owner was aware of the treatment group, assuring double-blinded conditions. Blinding was broken only by a statistician after completion of the 1st studies and data management. Blinded conditions were maintained throughout the 2 stages of follow-up for investigators, owners, and study monitors. Dogs kept the same study number throughout studies and so were maintained in the same treatment group; no reallocation was carried out.

Treatment

All dogs received conventional therapy, which included at least an ACEI. Furosemide was allowed after day 5 if necessary in the 3-month study and from at least 1 day before inclusion in the 2-month study. During the follow-up, veterinarians could change the dosage of, initiate, or terminate furosemide treatment. Therapy with digoxin, L-carnitine, or both also was allowed. In addition to this conventional therapy, dogs received either spironolactone or placebo. Spironolactone was given orally at the dosage of 2 mg/kg once a day with food. The dose actually achieved was 2.33 ± 0.34 mg/kg/d (mean \pm SD), according to the conclusions of the dose determination and pharmacokinetic studies.^{a,17} Scored tablets of 10, 40, and 80 mg were used.

Evaluation Schedule

In both 1st studies, examinations were planned on a similar schedule. Full clinical examination, thoracic radiography, electrocardiography, echocardiography, urine, and blood samples were performed on day 0. Clinical and radiographic examinations and blood sampling were performed during the 1st week, at day 28 (except radiographs), days 56 and 84. During the long-term study, clinical and radiographic examinations and blood sampling were performed at 3-month intervals. Echocardiographic examinations were undertaken at inclusion and after 6 and 12 months. Prospective data and safety variables will be described in a separate forthcoming paper.

Clinical Evaluation

The protocol included assessment of the clinical variables cough, dyspnea, exercise intolerance (eg, outside mobility, activity at home, attitude at the veterinary

practice) and syncope (Table 1). Lateral and dorsal thoracic radiographs were obtained to evaluate heart size (using the Buchanan VHS)¹⁶ and the presence of pulmonary edema. Standard echocardiography measurements were performed (M-mode measurements of LV size, 2D for diagnosis and measurements of LA/Ao ratio).¹⁵ Classification of the stage of HF at inclusion was assessed according to the ISACHC.¹⁸

Survival Evaluation

The primary end-point was cardiac-related death, euthanasia because of MR, or severe worsening of MR. Severe worsening was defined as the need to introduce an unauthorized cardiac therapy or to increase the dosage of furosemide over 10 mg/kg/d to prevent life-threatening CHF. Whenever the dog died spontaneously or was euthanized, the investigator specified whether it was considered that the cause of death was cardiac or non-cardiac and noted the precise reason. Cardiac mortality was assessed by pooling natural deaths and euthanasia owing to cardiac causes.

Data Management

Raw data were entered by 2 independent operators, automatically compared, and corrected according to investigators' answers to queries. The database was audited before it was finalized and transferred for statistical analyses. Data management and quality control were performed by Ceva Santé Animale according to internal GCP procedures.

Statistical Analyses

Analyses were carried out on SAS Institute Inc software version 9.1 and Stata version 8.0. All analyses were 2-tailed. Conditions of application were verified before by a statistical test.

For quantitative variables: normality of distribution, assumption of equal variances (F -test, t -test, Satterthwaite correction applied where appropriate).

For qualitative variables: Chi-square test or Fisher's exact test according to the expected frequencies.

Survival analysis: log rank test is a nonparametric test and, as such, does not require any distributional assumptions about the event times.

Cox model: assumption that the event hazard rate does change over time, but that the ratio of event hazards between 2 individuals is constant. This is known as the proportional hazards assumption, which requires that the ratio of hazards between any 2 values of a covariate not vary with time.

The descriptive analysis and initial comparability between treatment groups were made on the characteristics and clinical criteria recorded at inclusion in the 2 1st studies. The baseline data were compared between treatment groups, to check that owner withdrawals had not produced bias, and also between these 2 studies (study effect). For quantitative variables, the 2-sample t -test (Student's t -test) was used to compare the mean values. Assumption of equal variances was tested by the F -test.

Table 1. Base-line characteristics of 212 dogs with MMVD in the ITT population (mean \pm SD or number and percentage).

	Spironolactone (n = 102)	Control (n = 110)	<i>P</i> Value (Treatment Effect)	<i>P</i> Value (Study Effect)
Age (years)	11.2 \pm 3.3	11.5 \pm 2.4	.59	.39
Weight (kg)	11.1 \pm 9.6	11.6 \pm 9.1	.74	.15
Sex				
Male	64 (62.7%)	62 (56.4%)	.15	.36
Female	38 (37.3%)	48 (43.6%)		
Breeds ^a				
Poodle	19 (18.8%)	19 (17.3%)		
Dachshund	10 (9.9%)	7 (6.4%)		
Yorkshire Terrier	9 (8.9%)	13 (11.8%)	.67	.78
Cavalier King Charles	10 (9.9%)	6 (5.5%)		
Mixed breed	19 (18.8%)	30 (27.3%)		
Others	34 (33.7%)	35 (31.8%)		
Etiology ^a				
MMVD	94 (92.2%)	100 (90.9%)	.75	.76
MMVD + DCM	8 (7.8%)	10 (9.1%)		
ISACHC classification ^a				
Early stage II	24 (23.5%)	20 (18.4%)		
Stage II	65 (63.7%)	81 (74.3%)	.21	.32
Stage III	13 (12.8%)	8 (7.3%)		
Treatment before inclusion				
ACEI	102 (100%)	110 (100%)		
Benazepril	49 (48.0%)	62 (56.4%)		
Enalapril	21 (20.6%)	15 (13.6%)	.35	.46
Imidapril	7 (6.9%)	11 (10.0%)		
Ramipril	25 (24.5%)	22 (20.0%)		
Furosemide	42 (41.2%)	56 (50.9%)	.15	.25
Digoxin	5 (4.9%)	4 (3.6%)	.74	.49
Duration of cardiac treatment before inclusion				
ACEI (days)	290 \pm 381	298 \pm 477	.57	.0062
Furosemide (days)	169 \pm 265	137 \pm 294	.90	—
Cough				
None	5 (4.9%)	6 (5.5%)		
Occasional	44 (43.1%)	48 (43.6%)		
Frequent	48 (47.1%)	44 (40.0%)	.39	.82
Persistent	5 (4.9%)	12 (10.9%)		
Dyspnea				
None	31 (30.4%)	35 (31.8%)		
Moderate	66 (64.7%)	73 (66.4%)	.45	.49
Severe	5 (4.9%)	2 (1.8%)		
Syncope				
None	85 (83.3%)	99 (90.0%)		
< 4 per month	13 (12.8%)	11 (10.0%)	.085	.055
> 4 per month	4 (3.9%)	0 (0%)		
Mobility				
High	17 (16.7%)	19 (17.3%)		
Moderate	63 (61.8%)	68 (61.8%)		
Low	19 (18.6%)	21 (19.1%)	.96	.80
None	3 (2.9%)	2 (1.8%)		
Activity				
High	12 (11.8%)	16 (14.6%)		
Moderate	73 (71.6%)	79 (71.8%)		
Low	17 (16.7%)	14 (12.7%)	.61	.45
None	0 (0%)	1 (0.9%)		
Demeanour				
Normal	39 (38.2%)	49 (44.6%)	.47	.63
Reduced	60 (58.8%)	55 (50.0%)		
Minimal	3 (2.9%)	5 (4.6%)		
Inactive	0 (0%)	1 (0.9%)		
X-ray/Echocardiography				
Heart size (vertebra) ^a	11.8 \pm 1.4	11.7 \pm 1.1	.59	.015
LA/Ao ratio ^b	1.8 \pm 0.5	1.8 \pm 0.5	.48	.048

Table 1. (Continued).

	Spirolactone (n = 102)	Control (n = 110)	P Value (Treatment Effect)	P Value (Study Effect)
Biochemistry^c				
Creatinine (µmol/L)	80.6 ± 29.2	87.7 ± 36.2	.12	.0016
Urea (mmol/L)	8.6 ± 4.2	8.9 ± 6.9	.66	.0027
Potassium (mmol/L)	4.7 ± 0.5	4.6 ± 0.6	.70	.42

^aOne missing value.

^bTwo missing values.

^cThree missing values.

Mobility (outdoor): High, dog has strong desire and interest to go out for walks or play. Appears alert and responsive to surrounding environment. Moderate, dog has some interest and desire to go out for walks or play. Appears alert to the surrounding environment. Low, dog has low interest to go for a run or walk, does not play often, appears not very alert to the surrounding environment. None, dog has no interest to go for a walk neither to the surrounding environment, unable to walk short distances without developing dyspnea. **Activity (indoor):** High, dog moves around with ease, capable of climbing stairs or running short distances. The dog is alert, reacts to the environment, and responds to external stimuli. Moderate, dog has a tendency to be inactive, but gets up and moves around a few times per day. Has difficulty with stairs and is unable to cope with long walks. Still responsive to external stimuli. Low, dog remains inactive all day and only gets up to eat, drink, or urinate. Responds to stimuli with difficulty. None, dog will only get up or move if strongly encouraged, unable to walk short distances without developing dyspnea. **Demeanor (at the clinic):** Normal, usual activity displayed, dog alert and responsive. Reduced, activity moderately depressed, but the dog reacts to external stimuli, and gets up and moves around. Minimal, level of activity minimal, but the dog responds around it with difficulty and only gets up or moves with significant encouragement. Inactive, dog very depressed or hypoxic, does not move unless forced to.

MMVD, myxomatous mitral valve disease; DCM, dilated cardiomyopathy; ISACHC, International Small Animal Cardiac Health Council.

In case of rejection of homogeneity, the Satterthwaite correction was applied. For qualitative variables, chi-square test or Fisher's exact test was used in order to compare the frequencies between treatment groups. The percentage of morbidity-mortality or mortality events at the end of follow-up was compared between groups by Fisher's exact test. Survival curves were obtained by the Kaplan-Meier method. Survival analysis, allowing for censoring when dogs could no longer be observed, was performed by log rank test for comparing survival of the 2 treatment groups, and by a multifactorial Cox proportional hazard model to assess the impact of some covariates, such as study of origin, duration of cardiac treatment before inclusion (< or > median duration of 140 days), and prescription of furosemide (yes/no). The hazard ratio (HR) and its 95% confidence interval also were evaluated. Analyses of the homogeneity of the population were done from the intention to treat (enrolled treated population whatever the compliance to the study protocol) data set. Because reasons for nonadherence to the protocol may be related to prognosis and possibly introduce bias in survival analysis carried out on the per protocol population (study protocol strictly respected),¹⁹ survival analyses were performed both on the intention to treat and per protocol populations. Exclusions from the per protocol population were done under blinded conditions. Level of significance was set at *P* < .05. Values were reported as mean ± SD.

Results

Baseline Characteristics of Dogs at Inclusion

Two hundred and twenty-one dogs were enrolled. Only 7 dogs presented with DCM and these dogs were excluded from further analysis. Furthermore, 2 dogs were lost to follow-up before the 1st examination, which

left 212 dogs presenting with MMVD (intention to treat population). Among these 212 dogs, 22 were not retained for the per protocol analyses because of heart enlargement (VHS < 10.5) at inclusion time (3 in each group), inappropriate treatment before inclusion (3 and 1 in spironolactone and control groups, respectively), or poor compliance with the administration of tested treatments (7 and 5 in spironolactone and control groups, respectively). The corresponding per protocol population included 190 dogs. The baseline data are presented only for the 212 dogs in the intention to treat population and were comparable between the 2 treatment groups (Table 1, treatment effect). Because of the different study protocols of the 2 short-term studies, ie, furosemide was allowed in 1 study but not in the other, there were significant differences between studies (Table 1, study effect), but these differences did not produce any differences between treatment groups.

From the intention to treat population of 212 dogs with MMVD, 123 of the 179 dogs that completed the 1st studies continued into the 12-month study. The remaining 56 cases were not enrolled because the owners were not motivated to continue to adhere to the re-examinations required in the 12-month study protocol. These 56 cases were well balanced between the treatment groups (30/102 in the spironolactone group and 26/110 in the control group, *P* = .34). In the per protocol population, the outcome of dogs after the 1st studies was similar (Table 2).

Overall Outcome

Effect of Therapy on Outcome

In the spironolactone and control groups, respectively, 34.3% (35/102) and 40% (44/110) of dogs completed the 15-month period (Table 2). In the spironolactone group,

Table 2. Outcome during the 15-month follow-up for the 212 dogs in the intention-to-treat population and the 190 dogs in the per protocol population (in brackets).

	Spironolactone Group			Control Group		
	1st Studies	12-Month Study	Total n = 102 (88)	1st Studies	12-Month Study	Total n = 110 (102)
	6 (4) 4 (2) 0 2 (2) 40 (32) 10 (5) 4 (2) 0 2 (0) 1 (1) 3 (2) 30 (27)	5 (5) 2 (2) 0 3 (3) 51 (47) 16 (15) 8 (7) 2 (2) 0 1 (1) 5 (5) —	11 (9) 6 (4) 0 5 (5) 91 (79) 26 (20) 12 (9) 2 (2) 2 (0) 2 (2) 8 (7) 30 (27) 35 (32)	12 (11) 6 (6) 4 (3) 2 (2) 31 (28) 5 (4) 3 (3) 0 0 1 (0) 1 (1) 26 (24)	16 (15) 8 (8) 4 (4) 4 (3) 51 (48) 7 (6) 2 (2) 1 (1) 0 2 (2) 2 (1) —	28 (26) 14 (14) 8 (7) 6 (5) 82 (76) 12 (10) 5 (5) 1 (1) 0 3 (2) 3 (2) 26 (24) 44 (42)
Reached endpoint						
Cardiac death						
Euthanasia because of MR						
Severe worsening of MR						
Censored cases						
Premature withdrawal for other reasons than HF						
Concomitant diseases						
Car accident						
Deviation to protocol						
Lost to follow-up						
Owner's will						
Not enrolled in 12-month study by owner's will						
15-month follow-up completed						

10.8% (11/102) of dogs reached the primary end-point and 25.5% (28/110) in the control group (Fisher's exact test, $P = .0046$) (Table 3). Causes of withdrawals not related to HF included the owner's wish to stop after the 1st studies, concomitant disease, the owners moved away, and car accident. There were 54.9% (56/102) and 34.5% (38/110) in the spironolactone and control groups, respectively.

The estimated 15-month survival rate was 84% for the dogs treated with spironolactone and conventional therapy, and 66% for the dogs in the control group (log rank test, $P = .017$) (Table 3 and Fig 2). If only mortality (cardiac death and euthanasia related to MR) is considered, the 15-month survival rate was 92% in the spironolactone group and 73% in the control group (log rank test, $P = .0071$) (Table 3 and Fig 3).

Results from the per protocol population were similar (Tables 2 and 3).

Cox Proportional Hazard Models

The HR of treatment effect was 0.45 (95% confidence limits [CL], 0.22–0.90, $P = .023$). This represents a 55% reduction in the risk of morbidity-mortality (severe deterioration, natural death, or euthanasia related to MR). If only mortality (natural death and euthanasia related to MR) was considered, the HR was 0.31 (95% CL, 0.13–0.76, $P = .011$), which represented a significant 69% reduction in the risk of mortality. The survival data analysis therefore showed that spironolactone decreased morbidity-mortality to less than half and mortality to about one third of the control group (Figs 4, 5, Table 3).

The study of origin (2-month study or 3-month study) and the duration of cardiac treatment before day 0 did not affect the estimate of the HR of the treatment effect, its confidence interval, or its statistical significance (Figs 4, 5). The concomitant administration of furosemide (which was well balanced in both treatment groups) significantly decreased the survival probability (Figs 4, 5). It did not affect the estimate of the HR of the treatment effect. Results from the per protocol population were similar (Tables 2 and 3).

Discussion

Study Findings

This study demonstrated that the addition of spironolactone to conventional cardiac therapy significantly reduced the risk of cardiac morbidity and mortality in dogs with MMVD when compared with conventional therapy alone (ie, ACEI plus furosemide or digoxin, if needed). A 55% reduction in the risk of cardiac morbidity-mortality was found. A greater 69% reduction in the risk of mortality was found when only cardiac mortality was analyzed.

This difference between the treatment groups was based on a higher number of dogs reaching the endpoint in the control group (28/110) than in the spironolactone group (11/102), whereas the total number of dogs not completing the study for cardiac or other miscellaneous reasons (mainly dogs not enrolled in the 12-month study

Table 3. Hazard ratio, survival rates, events rates, and log rank test taking into account either morbidity-mortality events (cardiac death, euthanasia because of MR, and worsening of MR) or mortality events (cardiac death and euthanasia because of MR).

	Intention-to-Treat Population (n = 212)	Per Protocol Population (n = 190)
Effect on the primary endpoint (cardiac death, euthanasia because of MR, and worsening of MR)		
Hazard ratio ^a	0.45 [0.22–0.90]	0.40 [0.19–0.86]
Reduction in risk ^b	55%	60%
Estimated survival rate at 15 months (spiro. versus ref.)	84 versus 66%	85 versus 67%
Survival analysis (log rank test) <i>P</i> value	<i>P</i> = .017	<i>P</i> = .015
% “events” at end of follow-up (spiro. versus ref.) and Fisher exact test, <i>P</i> value	10.8 versus 25.5 <i>P</i> = .0046	10.2 versus 25.5 <i>P</i> = .0053
Effect on mortality (cardiac death and euthanasia because of MR)		
Hazard ratio ^a	0.31 [0.13–0.76]	0.22 [0.08–0.65]
Reduction in risk ^b	69%	78%
Estimated survival rate at 15 months (spiro. versus ref.)	92 versus 73%	93 versus 72%
Survival analysis (log rank test) <i>P</i> value	<i>P</i> = .0071	<i>P</i> = .0026
% “events” at end of follow-up (spiro. versus ref.) and Fisher exact test, <i>P</i> value	5.9 versus 20.0 <i>P</i> = .0019	4.6 versus 20.6 <i>P</i> = .0006

Survival rate was significantly higher in dogs treated with spironolactone. Likewise, the risks for reaching the mortality endpoint or the morbidity-mortality endpoint were significantly reduced in dogs treated with spironolactone. Results were similar in both intention-to-treat and per protocol populations.

^aHazard ratio and 95% confidence interval.

^bReduction in risk = 1-hazard ratio.

and also premature withdrawals for noncardiac reasons) was similar between treatment groups (67/102 and 66/110 in the spironolactone and control groups, respectively) (Table 2).

When the covariates were tested in the multifactorial Cox proportional hazard model, furosemide treatment was associated with a significant negative effect although the beneficial effect of spironolactone was not affected. In this analysis, the furosemide covariate was defined as prescription at any time during the study/no prescription at all, because it was impossible to analyze this covariate in detail (eg, dosage, duration) because of multiple changes in the dosage and, in some cases, discontinued furosemide therapy during the 15-month follow-up. One likely explanation for the higher morbidity-mortality and mortality probabilities, observed in both treatment groups when furosemide was prescribed, could be that

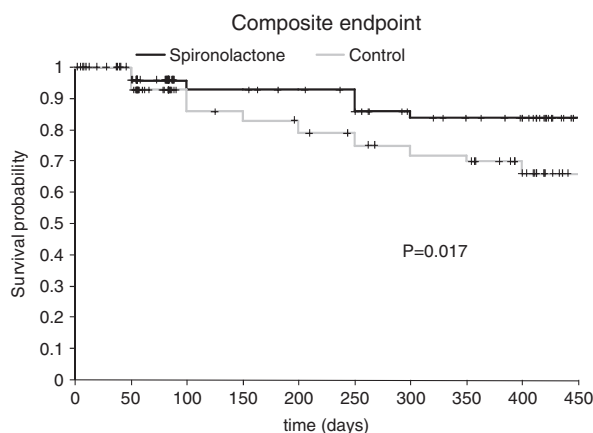


Fig 2. Kaplan-Meier plot of percentage of dogs remaining in the study as a function of time, in the 212 dogs in the intention-to-treat population. The endpoint was a composite of cardiac death, euthanasia because of mitral regurgitation (MR), and worsening of MR. Dogs treated with spironolactone had a significant longer time period in the study.

dogs receiving furosemide had more advanced disease at study inclusion.

It is possible to draw comparisons with other published survival studies on dogs with HF caused by MMVD, paying particular attention to the definition of endpoints, the severity and diagnosis of HF at entry and the concomitant cardiac treatments. The present study represents one of the largest field trials conducted on survival in dogs with moderate to severe MR caused by MMVD with 221 enrolled dogs versus 260, 162, and 110 in the QUEST,²⁰ BENCH,²¹ and LIVE²² studies, respectively. The baseline data and survival analyses indicate that the population included in this study consisted of a higher proportion of less advanced cases compared with the previously mentioned trials: 89.6% (190/212) of the cases with MMVD started the study at ISACHC class II

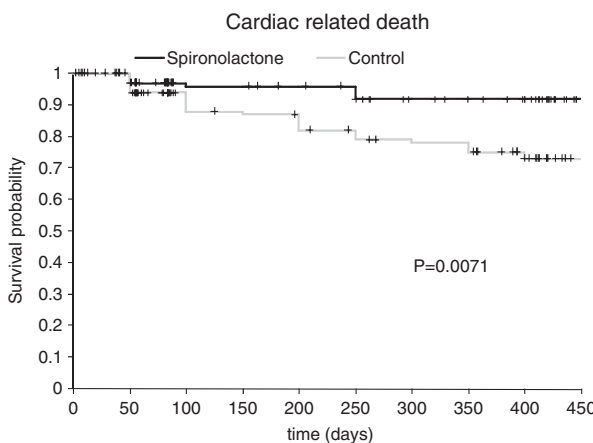


Fig 3. Kaplan-Meier plot of percentage of dogs remaining in the study as a function of time, in the 212 dogs in the intention-to-treat population when cardiac related death (sudden death or cardiac related euthanasia) was used as endpoint. Dogs with myxomatous mitral valve disease treated with spironolactone had a significant longer time period in the study.

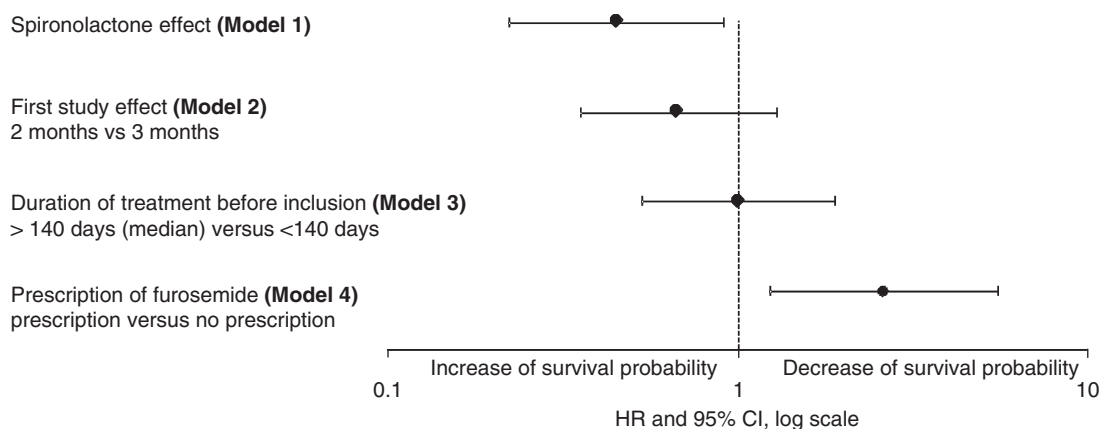


Fig 4. Hazard ratios (●) and 95% confidence interval (horizontal lines, log scale) obtained from the multifactorial Cox-proportional analysis of the 212 dogs in the intention-to-treat population. The endpoint was the composite endpoint of cardiac death, euthanasia because of mitral regurgitation (MR), and worsening of MR.

Cox proportional hazard model:

Model 1: Cox regression of treatment effect without any other variable.

Treatment group: HR = 0.45, 95% CI [0.22–0.90]; $P = .023$

Model 2: Cox regression of treatment effect and 1st study.

Treatment group: HR = 0.46; 95% CI [0.23–0.93]; $P = .031$

1st study HR = 0.67; 95% CI [0.35–1.28]; $P = .22$

The HR (0.46) is similar to the HR (0.45) before adjustment.

Model 3: Cox regression of treatment effect and duration of treatment before inclusion.

Treatment group: HR = 0.45; 95% CI [0.22–0.90]; $P = .023$

Duration of treatment before inclusion: HR = 1.00; 95% CI [0.53–1.88]; $P = .99$

The HR (0.45) is equal to the HR (0.45) before adjustment.

Model 4: Cox regression of treatment effect and Furosemide treatment.

Treatment group: HR = 0.46; 95% CI [0.23–0.93]; $P = .031$

Furosemide treatment: HR = 2.56; 95% CI [1.22–5.44]; $P = .013$

The HR (0.46) is similar to the estimate before adjustment.

Treatment effect: the hazard ratio is estimated as 0.45 or 0.46 in all models with $P = .023$ – $.031$ with 95% CI around 0.22–0.93. Spironolactone treatment significantly associated with a risk reduction for reaching the endpoint.

as compared with 67% of the BENCH cases, 0 and 89% at New York Heart Association classes II and III, respectively, in the LIVE study and mainly advanced stages in the QUEST study. Thus, the estimated survival rate (morbidity-mortality) is 66% in our control group at 15 months versus 49% in the benazepril group at 12 months (BENCH Study). The rate of premature withdrawals for cardiac reasons (severe deterioration and death) was 25.5% in our control group at 15 months versus 48.1% in the enalapril group at 17 months (LIVE Study). The number of patients reaching the endpoints in the present study was limited by dogs withdrawn for other reasons, predominantly the owner's wish to stop after the 1st studies. In most previous survival studies involving CHF in dogs, median survival times were calculated. Because <50% of the population reached the end-point in the present study, these data could not be calculated. However, the best way to estimate the difference between 2 survival curves is the estimation of the HR, which compares the chance that a member of each population will have an event at any given time. This is the usual statistical way to report results of survival analyses in the human medical literature. It was assessed only in the present study and in the BENCH and QUEST studies. The BENCH study showed that benazepril decreased the risk of severe or lethal deterioration by 44% after 18 months. In the present study, all dogs

were receiving an ACEI, yet spironolactone led to a 55% reduction in risk of cardiac death, euthanasia because of MR or worsening of MR during a 15-month follow-up, demonstrating that the benefit of spironolactone treatment is additional to that derived from the use of ACEI alone. Because the QUEST study is a positive-controlled study, the HR cannot be compared with the results from placebo-controlled studies.

If we consider the reduction of the risk of mortality from cardiac causes, the results in dogs were more obvious with a 69% reduction at 15 months with spironolactone which compares favorably to the 31% reduction observed at 3 years in the RALES in human medicine.¹³

Suggested Mode of Action for Spironolactone

The beneficial effects of spironolactone on survival may not only be explained by the diuretic effect of the drug. It has been shown in rats^{4,23–25} and human^{3,26} patients that aldosterone induces myocardial and perivascular fibrosis and alters the endothelial function of vessels.^{12,27} Studies performed in human patients with CHF showed that these effects are counteracted by aldosterone antagonists.^{27,28} Studies in experimental dogs with MR caused by chordal sectioning showed that the process of myocardial remodeling in response to MR is characterized by

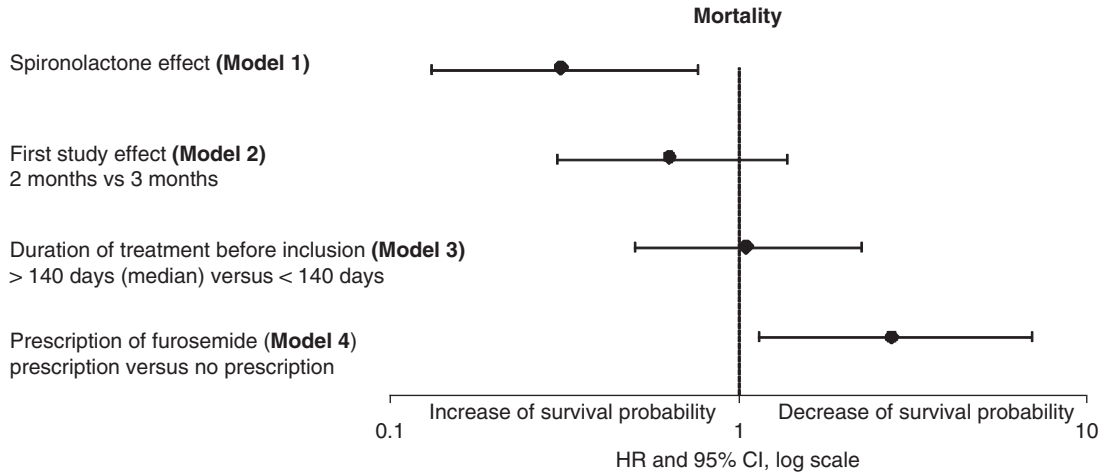


Fig 5. Hazard ratios (●) and 95% confidence interval (horizontal lines, log scale) obtained from the multifactorial Cox-proportional analysis in the 212 dogs in the intention-to-treat population when cardiac related death (sudden death or euthanasia because of mitral regurgitation) was used as end point.

Cox proportional hazard model:

Model 1: Cox regression of treatment effect without any other variable.

Treatment group: HR = 0.31; 95% CI [0.13–0.76]; *P* = .011

Model 2: Cox regression of treatment effect and 1st study.

Treatment group: HR = 0.32; 95% CI [0.13–0.79]; *P* = .014

1st study: HR = 0.63; 95% CI [0.30–1.36]; *P* = .24

The HR (0.32) is similar to the HR (0.31) before adjustment.

Model 3: Cox regression of treatment effect and duration of treatment before inclusion.

Treatment group: HR = 0.31; 95% CI [0.13–0.76]; *P* = .011

Duration of treatment before inclusion: HR = 1.07; 95% CI [0.51–2.23]; *P* = .88

The HR (0.31) is equal to the HR (0.31) before adjustment.

Model 4: Cox regression of treatment effect and Furosemide treatment.

Treatment group: HR = 0.32; 95% CI [0.13–0.80]; *P* = .014

Furosemide treatment: HR = 2.78; 95% CI [1.13–6.86]; *P* = .023

The HR (0.32) is similar to the estimate before adjustment.

Treatment effect: the hazard ratio is estimated as 0.31 or 0.32 in all models with *P* = .011–.014 with 95% CI around 0.13–0.80. Spirolactone treatment significantly associated with a risk reduction for reaching the end point.

a loss of interstitial collagen, a process that is believed to be mediated by metalloproteinases.²⁹ The loss of collagen weave surrounding the myocytes allows myocyte slippage, which is important for cardiac dilatation. These experimental studies involved healthy comparably young dogs in which MR was induced. The population dogs with naturally occurring MMVD and MR are middle aged to old dogs, and the experimental models may not completely mimic the situation in these dogs. Recent literature demonstrated that a proportion of dogs with naturally occurring MMVD had intramyocardial arterial changes that were associated with areas of fibrosis in the myocardium, the so-called replacement fibrosis.³⁰ The same authors suggested that more severe intramyocardial arteriosclerotic changes and more severe replacement fibrosis shorten survival time from the onset of cardiac therapy to cardiac death or euthanasia.³¹ An antifibrotic effect also has been described in intracoronary microembolization and rapid ventricular pacing canine models of CHF with spironolactone and eplerenone, another aldosterone antagonist.^{32,33} The model of rapid ventricular pacing induced volume overload in cardiac chambers.³² That model showed a correlation between the increase of the interstitial collagen volume fraction and conduction abnormalities in the right atrium. In that study, spironolactone (15 mg/kg) had a significant beneficial effect on

both fibrosis development and conduction changes in the right atrium compared with the control group. In the model of microembolization, a 3-month treatment with eplerenone significantly decreased the volume fraction of reactive interstitial fibrosis and decreased the amount of replacement fibrosis in the myocardium.³³ That model induced a left ventricle end-diastolic wall stress but in a completely artificial way compared with spontaneous occurrence in dogs.

We believe that the observed beneficial effect of spironolactone on survival time in the present study could partly be related to a counteractive effect of spironolactone on the arterial changes and replacement fibrosis.

Study Limitations and Further Investigations

Because of the low event rate, we could not include all covariates of interest in the same analysis, but had to examine the effects of each singly. Because none of the covariates had any effect on the HR, we believed that the HR would be unchanged and would remain significant were we able to include all in a single analysis. The loss of follow-up because owners did not wish their dogs to continue to the longer study increased the number of censored cases to a significant extent (32.4% of censored cases, 56/173, Table 2).

The type of ACEI was not controlled in the present study, with the exception that they had to be licensed within the European Union.

To assure homogeneity of the groups and also because pimobendan was not widely used in these dogs in the countries in which the centers were located at time of the planning (2002–2003), conventional therapy was defined as an ACEI \pm furosemide \pm digoxin. However, pimobendan is increasingly prescribed and has been shown to extend survival in dogs with CHF because of MMVD compared with an ACEI.^{20,34} Spironolactone was part of the treatment failure endpoint in the QUEST study but the study was not designed to investigate the interaction of the combined use of the substances. Further studies are warranted to assess the clinical benefit of pimobendan when combined with spironolactone. Also, the small size of the DCM population did not permit a separate analysis. The benefit of spironolactone in DCM should be more deeply investigated.

In conclusion, this clinical trial of spironolactone therapy over a 15-month period in dogs with moderate to severe MR caused by MMVD demonstrated a beneficial effect of spironolactone when added to conventional therapy. The risk of cardiac-related death or euthanasia was reduced by 69%, as compared with conventional therapy alone (ie, ACEI plus furosemide or digoxin, if needed). This finding supports spironolactone as part of the treatment protocol in dogs with MMVD.

Footnotes

^a Scientific discussion of Prilactone, European Medicine Agency, 2007

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