

Lack of efficacy of low-dose spironolactone as adjunct treatment to conventional congestive heart failure treatment in dogs

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Aldosterone plays an important role in the pathophysiology of heart failure. Aldosterone receptor blockade has been shown to reduce morbidity and mortality in human patients with advanced congestive left ventricular heart failure. This study was designed to assess the efficacy and tolerance of long-term low-dose spironolactone when added to conventional heart failure treatment in dogs with advanced heart failure. Eighteen client-owned dogs with advanced congestive heart failure due to either degenerative valve disease ($n = 11$) or dilated cardiomyopathy ($n = 7$) were included in this prospective, placebo-controlled, double-blinded, randomized clinical study. After initial stabilization including furosemide, angiotensin-converting enzyme inhibitors, pimobendan and digoxin, spironolactone at a median dose of 0.52 mg/kg (range 0.49–0.8 mg/kg) once daily ($n = 9$) or placebo ($n = 9$) was added to the treatment, and the dogs were reassessed 3 and 6 months later. Clinical scoring, echocardiography, electrocardiogram, systolic blood pressure measurement, thoracic radiography, sodium, potassium, urea, creatinine, alanine aminotransferase, aldosterone and aminoterminal atrial natriuretic propeptide were assessed at baseline, 3 and 6 months. Survival times were not significantly different between the two treatment groups. Spironolactone was well tolerated when combined with conventional heart failure treatment.

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INTRODUCTION

In humans and animals with advanced congestive heart failure, serum aldosterone concentrations are increased as a result of the activation of the renin–angiotensin–aldosterone system (RAAS) (Swedberg *et al.*, 1990). Aldosterone plays a key role in the pathophysiology of congestive heart failure. It promotes the retention of sodium, the loss of magnesium and potassium, sympathetic activation, parasympathetic inhibition, myocardial and vascular remodelling, baroreceptor dysfunction and impairs arterial compliance (Struthers, 2004). The actions of aldosterone, although beneficial in the short term, contribute to the progression of heart failure in the long term (Katz, 2003). Treatment with angiotensin conversion enzyme (ACE) inhibitors reduces angiotensin II and aldosterone production and has been shown to be of great clinical benefit in the treatment of heart failure in humans (CONSENSUS study, 1987) and dogs (COVE study, 1995; IMPROVE study, 1995; Ettinger *et al.*, 1998;

BENCH study, 1999). However, in up to 45% of human patients with heart failure, serum aldosterone and/or angiotensin II concentrations are elevated despite treatment with ACE inhibitors, a phenomenon called ‘aldosterone escape’ (MacFadyen *et al.*, 1999; Van de Wal *et al.*, 2006). Treatment with ACE inhibitors may therefore not be sufficient to control aldosterone production, and concurrent aldosterone receptor blockade may be beneficial. The ‘Randomised Aldactone Evaluation Study’ (RALES), a large, multicentre study involving 1663 human patients, showed that spironolactone, when added in a low dose (25 mg/day), which will not lead to significant diuresis, to conventional treatment with ACE inhibitors, diuretics and cardiac glycosides, significantly reduces morbidity and mortality in human patients with advanced left ventricular dysfunction (Pitt *et al.*, 1999). The beneficial effect of spironolactone on survival was most pronounced in patients with elevated serum concentrations of procollagen III aminoterminal peptide (PIIINP), a serum marker of increased extracellular matrix

turnover. It was suggested that spironolactone acts by blunting aldosterone-induced myocardial remodelling, in particular myocardial fibrosis (Zannad *et al.*, 2000). Further subgroup studies showed a significant decrease in plasma brain natriuretic peptide (BNP) and N-terminal peptide of the aminoterminal atrial natriuretic propeptide (NT-proANP) as well as a significant increase in plasma angiotensin II and aldosterone in patients treated with low-dose spironolactone, indicating that, at this dose, spironolactone induced significant neurohormonal changes that could be responsible for the beneficial effect on morbidity and mortality (Rousseau *et al.*, 2002).

However, combined treatment with ACE inhibitors and spironolactone has been a concern in humans, especially for patients with reduced renal function, given that both drugs work synergistically via reduction in aldosterone secretion (ACE inhibitors) and inhibition of aldosterone binding to its receptor (spironolactone) and thus can lead to life-threatening hyperkalaemia (Berry & McMurray, 2001; Juurlink *et al.*, 2004).

A recent clinical trial showed a significant decrease in mortality in dogs with moderate to severe mitral valve disease when spironolactone was added at 2 mg/kg once daily (Bernay *et al.*, 2010). Concurrent treatment with an ACE inhibitor and spironolactone was well tolerated in that study.

The aims of the present randomized, prospective, double-blinded, placebo-controlled clinical study were firstly to assess the efficacy of long-term treatment with a low dose of spironolactone in addition to conventional treatment including furosemide, ACE inhibitors, pimobendan and digoxin on survival and on clinical, biochemical and neuroendocrine parameters in dogs with advanced heart failure due to either degenerative valve disease (DVD) or dilated cardiomyopathy (DCM) and secondly to evaluate its tolerance in this category of patients. The dose of spironolactone used in this trial (0.5–0.8 mg/kg once daily) was chosen to be clearly below the approved diuretic dose in dogs (2–4 mg/kg once daily) to show that potential positive effects of spironolactone are due to other than diuretic effects.

MATERIAL AND METHODS

The study was designed as a prospective, randomized, double-blinded placebo-controlled clinical trial. It was performed at the Department of Small Animal Clinical Studies of the Faculty of Veterinary Medicine, University of Liège (Belgium). The study protocol was approved by the ethics committee for animal research of the University of Liège (Belgium). Informed consent was obtained from each owner before enrolment of the animal in the study protocol.

Animals

Eighteen client-owned dogs of various breeds were included. Inclusion criteria were presence of advanced heart failure [Classes II and III according to the Scandinavian modified New York Heart Association system (NYHA); Table 1] secondary to either DVD or DCM, absence of severe concurrent disease and

Table 1. Scandinavian modified New York Heart Association (NYHA) system for classification heart failure stage in dogs with mitral valve disease

NYHA class	Clinical findings
I	Asymptomatic dogs with murmurs but no cardiac enlargement.
II	Asymptomatic dogs with murmurs and cardiac enlargement but no pulmonary oedema or congestion.
III	Slightly or moderately symptomatic dogs (dyspnoea, increased heart rate and disappearance of sinus arrhythmia) with murmurs, cardiac enlargement and interstitial pulmonary oedema.
IV	Severely symptomatic dogs with murmurs, cardiac enlargement and alveolar pulmonary oedema.

clinically stable heart disease for a minimum of 4 weeks on conventional treatment. Accepted conventional treatments were furosemide, ACE inhibitors (benazepril or enalapril), pimobendan and digoxin. Exclusion criteria were uncontrolled congestive heart failure despite conventional treatment, severe concurrent extra-cardiac disease and need for spironolactone at diuretic dose (2–4 mg/kg/day). Initial diagnosis and staging were based on history, clinical findings, electrocardiogram (ECG), thoracic radiography and echocardiography. Haematology and serum biochemistry (not shown) were performed in all patients as part of the initial work up.

Treatment groups

The dogs were paired in order of admission to the study and assigned in either of two treatment groups: Group 1 received spironolactone at a median dose of 0.52 mg/kg once daily (range 0.5–0.8 mg/kg); Group 2 received a placebo (vehicle tablets) in addition to conventional treatment. Spironolactone (2 mg) and placebo tablets were both prepared by the Department of Pharmacology of the Centre Hospitalier Universitaire of the University of Liège and were identical in appearance. The tablets were administered per mouth with food. The study was double-blinded, with the investigators and the owners unaware of treatment group assignment of individual dogs.

Clinical evaluation

The dogs included in the study were scheduled for evaluation 4 weeks after the start of the initial stabilization period (baseline) and after 1 week, and 3 and 6 months of treatment with either spironolactone or placebo. At baseline, 3 and 6 months dogs were evaluated by history, physical examination, indirect systolic blood pressure measured by Doppler method, standard six-lead ECG for 5 min, right lateral and dorsoventral thoracic radiography and echocardiography. Plasma sodium, potassium, urea, creatinine and alanine aminotransferase (ALT) were measured. At the 1-week visit, a history and physical examination were

performed and a blood sample was taken for measurement of sodium, potassium, urea and creatinine. The clinical variables were assessed using a scoring system widely used in veterinary clinical cardiac trials (IMPROVE study, 1995; Smith *et al.*, 2005; Table 2). Additional historical parameters were the following: presence or absence of syncope, vomiting, diarrhoea, polydipsia and polyuria. Parameters assessed during physical examination were body weight, colour of mucous membranes, capillary refill time, presence of a jugular pulse or jugular distension, dyspnoea, pulse rate and quality, the presence of a precordial thrill, rectal temperature, heart rate, respiratory rate, murmur grade, intensity, radiation and point of maximum intensity and respiratory noises.

Indirect systolic blood pressure measurement was performed using an ultrasonic Doppler flow detector (Parks Medical Electronics, Aloha, OR, USA) with all dogs in lateral recumbence. Either the metacarpal or metatarsal artery was used while the paw was held at the level of the right atrium. Cuff size and location used were recorded, and the same technique was maintained for each individual dog for all consecutive visits. A total of four measurements were performed. The first measurement was discarded, and the mean systolic blood pressure was calculated as a mean of the following three measurements.

A standard 6-lead ECG (Spacelabs Burdick, Deerfield, WI, USA) was performed with patients positioned in right lateral recumbency. The ECG trace was assessed for heart rate, waveforms, intervals and the presence or absence of arrhythmias. Thoracic

radiographs were assessed for size of the cardiac silhouette, chamber enlargements and signs of pulmonary congestion. The size of the heart was measured using the vertebral heart size (VHS), which is a number that normalizes heart size to body size using mid-thoracic vertebrae as units of measure (Buchanan & Bücheler, 1995). Others have called this vertebral heart system.

Haemodynamic evaluation

Two-dimensional, M-mode and Doppler echocardiography were performed without sedation using a 7.5, 5.0 and 2.5 MHz transducer (Esaote Megas, Pie Medical Equipment, Maastricht, the Netherlands). Three measurements were made for each variable, and the average value was used in the statistical analysis. Simultaneous ECG was performed during the echocardiographic examination. Two-dimensional and M-mode variables were determined in accordance with the guidelines established by the American Society of Echocardiography (Schiller *et al.*, 1989; O'Grady *et al.*, 2005). All echocardiographic parameters measured are listed in Table 5.

NT-proANP and aldosterone

At baseline, 3- and 6-month blood samples were collected in iced 5-mL polyethylene tubes containing EDTA (25 μ mol) and aprotinin (Aprotinin, Trasylol®; Bayer, Leverkusen, Germany) for measurement of NT-proANP and in dry tubes for measurement

Table 2. Scoring system used for clinical variables evaluated in dogs with heart failure (Amended from The Improve Study Group, 1995)

Cough scores	Respiratory effort	Appetite
0 – None	0 – Normal	1 – Increased
1 – Occasional	1 – Mildly increased effort	2 – Normal
2 – Frequent	2 – Laboured	3 – Decreased
3 – Persistent	3 – Respiratory distress	4 – Markedly decreased
Mobility	Attitude	Activity
1 – Very good: walks well, runs, capable of some strenuous activity.	1 – Increased: has stronger desire and interest than in the past to go out for walks or play with owner. Appears more alert and responsive to surrounding environment.	1 – High: moves around with ease, capable of climbing stairs or running short distances. Alert and responsive to external stimuli.
2 – Good: walks well, will run a short distance or pulls on lead but unable to do strenuous activity, tires easily after walking 1–2 blocks.	2 – Remained the same: has approximately the same degree of interest and desire to go out for walks or play with the owner as in the past, is more alert to the surrounding environment as before.	2 – Moderate: tends to be inactive but moves around a few times per day. Has difficulty with stairs and avoids long walks.
3 – Moderate: will walk, but for a limited distance before needing to rest.	3 – Decreased: has some interest, but plays less often, and has decreased interest to go for a run or walk.	3 – Low: generally inactive, tendency to remain in one place most of the day and is unable to climb stairs or walk more than short distance.
4 – Poor: can only walk a few yards before needing to rest.		4 – Minimal: remains inactive all day and only gets up to eat, drink or urinate.
5 – Very poor: to get up and move is a major effort, only able to move a few steps before resting.		5 – Incapacitated: will only get up or move if strongly encouraged by owner.

of aldosterone. After centrifugation within 30 min ($2000 \times g$, 10 min, 4°C), serum and plasma samples were frozen at -20°C and run batch wise at the end of the trial using radioimmunoassays validated for use in canine plasma (Aldosterone RIA: Coat-a-count TKAL 1,2; Diagnostic Products Corporation, Los Angeles, CA, USA).¹ All measurements were performed in duplicate. For aldosterone, the antiserum used had a cross-reactivity of 100% for aldosterone and of $<0.03\%$ for other compounds. The lowest limit of detection was 30.5 pmol/l and the coefficient of variation was 7.7%. For NT-proANP, the antiserum used had a cross-reactivity of 100% for human NT-pro-ANP1-126 and NT-proANP 1-98 and a cross-reactivity of $<0.01\%$ for other natriuretic peptides. The lowest limit of detection was 0.03 fmol/l and the coefficient of variation in the study was 5.5%.

Endpoints

Predefined endpoints were end of the trial, euthanasia, death or discontinuation of the trial as a direct consequence of heart failure and discontinuation of the trial for reasons other than worsening of heart failure.

Statistical analysis

The survival curves of the two groups were determined by the Kaplan–Meier method and were compared by the log-rank test and the Cox proportional hazard model.

The results were expressed as mean \pm standard deviation (SD) for quantitative variables. Baseline characteristics of the two treatment groups were compared to assess the level of balance between the two groups: the Wilcoxon rank-sum test was used to compare quantitative variables, while the chi-squared test was used for qualitative variables. To study the evolution of the echocardiographical and biochemical parameters over time, generalized linear mixed models were used, including the baseline overall clinical score as covariate to account for baseline difference between the two groups. Calculations were always carried out on the maximum number of data available. Missing data were not replaced. Results were considered to be significant at the 5% critical level ($P < 0.05$). Statistical analysis was carried out using SAS (version 9.1 for Windows, SAS Institute Inc., Cary, NC, USA) and S-Plus (version 9.0, TIBCO Software Inc., Palo Alto, CA, USA) software packages.

Power calculation was performed after the end of the trial and was based on the mean survival times observed in the study (Lachin & Foulkes, 1986). Power calculation was not performed pretrial because specific survival data needed to formulate a reasonable assumption were not available and extrapolations

from the human RALES trial (Pitt *et al.*, 1999) were considered inappropriate.

RESULTS

Animals

A total of 18 dogs were enrolled in the study between February 2003 and October 2004. Breeds represented were Bichon Frise ($n = 3$), Labrador retriever ($n = 2$), mixed breeds ($n = 3$) and 1 each of the following: Miniature Poodle, Maltese terrier, Wire-haired Dachshund, Miniature Pinscher, Rough Collie, German Shepherd, Boxer, Newfoundland, Whippet and Rottweiler.

Baseline characteristics

Baseline characteristics of the dogs enrolled in the study are presented in Table 3. At baseline, the overall clinical score was significantly higher in the spironolactone group than in the control group (7.1 ± 1.8 vs. 5.2 ± 1.6 , $P < 0.05$), because of higher mobility and activity scores. Atrial fibrillation was present in three dogs from the spironolactone group but only 1 dog in the control group. Mean serum potassium, while being within the normal range in all dogs, was significantly higher in the spironolactone group at baseline (4.4 ± 0.6 vs. 3.8 ± 0.3 mmol/l, $P < 0.05$).

Progression of clinical and haemodynamic parameters, NT-proANP and aldosterone

No adverse events were observed in the study population over the study period of 6 months. Progression of clinical, haemodynamic, biochemical and neurohumoral parameters in the two treatment groups during the study is presented in Tables 4 and 5. No significant difference was found between the two treatment groups with regard to the clinical score. The overall clinical score significantly increased in dogs with DCM but not in dogs with DVD over time ($P < 0.01$). Mean heart failure class increased over time in both treatment groups ($P < 0.01$). Dogs in the control group had a higher risk of moving into a higher class of heart failure than dogs in the spironolactone group ($P < 0.05$). Mean NT-proANP concentration increased significantly over time in the spironolactone group and significantly decreased in the control group ($P < 0.0001$). Plasma aldosterone concentrations ranged between 0 and 461 pmol/l (mean 135 ± 106 pmol/l). There was no significant difference between groups and no significant changes over time; however, only a small number of samples was available for assessment at 6 months.

Outcome – endpoints

There was no significant difference between the two treatment groups regarding survival (Fig. 1). The survival probability decreased with increasing doses of furosemide ($P < 0.05$). There was no effect of pimobendan on the survival probability ($P > 0.05$).

¹NT-proANP •Biotop (Medipolis center, Kivihartjuntie 11, Fin-90220 OULU – Finland), code BT02-100 tubes. The antibody employed in this kit is directed to the amino acid residues 80–96 of the human ANP sequence, which is identical to that observed in canine and feline NT-proANP over that region.

Table 3. Baseline characteristics of the 18 dogs included in the study. Results are reported for the entire population ($n = 18$) and according to the treatment group (spironolactone/placebo)

Characteristics	Total $n = 18$	Spironolactone $n = 9$	Placebo $n = 9$	P
Age (years)	9.6 ± 2.2	9.7 ± 1.7	9.6 ± 2.7	0.89
Sex				
Male (all entire)	11	7	4	0.15
Female (entire/neutered)	7	2 (1/1)	5 (2/3)	
Body weight (kg)	23.2 ± 18.4	29.7 ± 20.9	16.7 ± 13.7	0.17
Pathology				
DVD	11	6	5	0.63
DCM	7	3	4	
Clinical score				
Cough	0.6 ± 0.6	0.7 ± 0.7	0.4 ± 0.5	0.52
Dyspnoea	0.3 ± 0.5	0.2 ± 0.4	0.4 ± 0.5	0.35
Appetite	2.1 ± 0.5	2.1 ± 0.8	2.0 ± 0.0	0.99
Attitude	1.9 ± 0.6	2.0 ± 0.7	1.8 ± 0.4	0.46
Mobility*	1.8 ± 0.7	2.2 ± 0.7	1.4 ± 0.5	0.021
Activity*	1.5 ± 0.6	1.9 ± 0.6	1.1 ± 0.3	0.01
Overall score*	6.2 ± 2.0	7.1 ± 1.8	5.2 ± 1.6	0.047
Syncope	1	1	1	0.99
Vomiting/diarrhoea	4	1	3	0.26
PU/PD	13	6	7	0.60
Clinical parameters				
Increased respiratory effort	0.22 ± 0.44	0.33 ± 0.50	0.28 ± 0.46	0.62
Respiratory rate (breaths/min)	30.4 ± 24.8	24.6 ± 20.4	36.3 ± 28.5	0.37
Heart rate (beats/min)	133.2 ± 27.5	139.8 ± 30.7	126.7 ± 23.8	0.23
Heart failure stage				
II	12	5	7	0.23
III	6	4	2	
IV		–	–	
Systolic blood pressure (mmHg)	138 ± 33	152 ± 36	121 ± 20	0.10
VHS	11.8 ± 1.2	11.3 ± 0.8	12.3 ± 1.3	0.11
Serum biochemistry				
Urea (mg/dL)	24.6 ± 10.7	22.9 ± 5.8	26.5 ± 14.7	0.96
Creatinine (mg/dL)	1.35 ± 0.38	1.33 ± 0.44	1.36 ± 0.34	0.50
ALT (U/L)	92.8 ± 95.6	75.7 ± 54.7	112 ± 129	0.70
Sodium (mmol/l)	147.4 ± 3.7	147.1 ± 3.7	147.6 ± 4.0	0.99
Potassium (mmol/l)*	4.1 ± 0.6	4.4 ± 0.6	3.8 ± 0.3	0.042
Neuroendocrine parameters				
NT-proANP (fmol/l)	0.81 ± 0.46	0.70 ± 0.41	0.95 ± 0.51	0.63
Aldosterone (pmol/l)	139 ± 116	147 ± 144	129 ± 76	0.63
Number of dogs receiving medication				
Furosemide [†] (n)	17	8	9	0.30
Mean Furosemide dose (mg/kg/day)	4.2 ± 2.1	4.0 ± 1.7	4.4 ± 2.4	0.85
ACE inhibitor				
Benazepril [‡] (n)	15	8	7	0.53
Enalapril [‡] (n)	3	1	2	0.53
Pimobendan [§] (n)	9	4	5	0.64
Digoxin [¶] (n)	2	1	1	0.99

Significantly different results are indicated with an asterisk (*); [†]Furosemide EG; Eurogenerics, Omega Pharma, Brussels, Belgium; [‡]Fortecor[®]; Novartis Animal Health, Brussels, Belgium; [§]Enacard[®]; Merial, Anderlecht, Belgium; [¶]Vetmedin[®]; Boehringer Vetmedica GmbH, Ingelheim, Germany; [¶]Lanoxin[®]; GlaxoSmithKline, Genval, Belgium; DVD, degenerative valve disease; DCM, dilated cardiomyopathy; PU/PD, polyuria and polydipsia; VHS, vertebral heart size; ALT, alanine aminotransferase; NT-proANP, aminoterminal atrial natriuretic propeptide; ACE inhibitor, angiotensin-converting enzyme inhibitor.

Power calculation

The results of the power calculation are shown in Fig. 2. The statistical power of the study was 80% to identify a difference by 85% between the hazards in the treated and the placebo groups over a 24-month follow-up period.

To identify a survival difference of 70% between control and treatment groups with an 80% statistical power over a 24-month period, a minimum of 33 dogs per group would be needed. With a survival difference between groups of 20%, 190 dogs per group would need to be included to show a significant difference over a 24-month period.

Table 4. Progression of clinical, biochemical, neurohumoral markers over the 6-month study period in the two treatment groups

Parameter	Group	n	Baseline	n	3 months	n	6 months
Clinical score							
Cough	S	9	0.7 ± 0.7	7	0.7 ± 0.8	6	0.7 ± 0.5
	P	9	0.4 ± 0.5	7	0.7 ± 0.8	5	1.0 ± 1.4
Dyspnoea	S	9	0.2 ± 0.4	7	0.1 ± 0.4	6	0 ± 0
	P	9	0.3 ± 0.5	7	0.3 ± 0.5	6	1.2 ± 1.2
Appetite ^(a)	S	9	2.1 ± 0.8	7	2.1 ± 0.7	5	2.2 ± 0.5
	P	9	2.0 ± 0.0	7	1.9 ± 0.7	5	2.8 ± 1.1
Attitude ^(a)	S	9	2.0 ± 0.7	7	1.6 ± 0.5	6	2.0 ± 0.6
	P	9	1.8 ± 0.4	7	2.0 ± 0.6	4	1.8 ± 1.0
Mobility	S	9	2.2 ± 0.7	7	2.1 ± 0.4	6	2.0 ± 0.6
	P	9	1.4 ± 0.5	7	1.6 ± 0.5	4	2.3 ± 1.3
Activity	S	9	1.9 ± 0.6	7	1.6 ± 0.5	6	1.8 ± 0.8
	P	9	1.1 ± 0.3	7	1.1 ± 0.4	4	1.5 ± 1.0
Overall score ^(a)	S	9	7.1 ± 1.8	7	7.0 ± 1.2	6	6.5 ± 2.4
	P	9	5.2 ± 1.6	7	5.6 ± 1.5	6	6.8 ± 2.9
Other clinical findings							
Syncope	S	9	1	6	0	6	0
	P	9	1	7	0	6	0
Vomiting/diarrhoea	S	9	1	7	1	6	0
	P	9	3	7	2	6	1
PU/PD	S	9	6	7	6	6	6
	P	9	7	7	6	6	5
Increased respiratory effort	S	9	0.2 ± 0.4	7	0.1 ± 0.6	6	0.0 ± 0.0
	P	9	0.3 ± 0.5	7	0.3 ± 0.5	6	1.2 ± 1.2
Respiratory rate	S	9	24.6 ± 20.4	7	18.4 ± 18.2	6	6.2 ± 12.7
	P	9	36.3 ± 28.5	6	22.8 ± 26.7	5	51.0 ± 40.3
Heart rate	S	9	140 ± 30.7	7	116 ± 24.5	6	119 ± 11.7
	P	9	127 ± 238	7	122 ± 24.8	6	130 ± 301
Electrocardiogram heart rate ^(a)	S	7	134 ± 22.2	6	128 ± 26.4	5	120 ± 7.1
	P	9	146 ± 27.4	7	143.4 ± 38.9	5	131 ± 26.0
Heart failure stage^{(a),(b)}							
II	S		5/9		3/6		3/5
III	S		4/9		2/6		2/5
IV	S		0/9		1/6		0/5
II	P		7/9		5/6		1/5
III	P		2/9		1/6		3/3
IV	P		0/9		0/6		2/3
Blood pressure	S	8	152 ± 36	6	148 ± 29	4	141 ± 15
	P	7	121 ± 19	4	122 ± 36	4	151 ± 32
VHS	S	8	11.3 ± 0.8	6	12.0 ± 0.8	5	11.6 ± 0.8
	P	8	12.3 ± 1.3	6	12.2 ± 0.9	6	12.3 ± 1.4
Plasma biochemistry							
Urea (mg/dL)	S	9	22.9 ± 5.8	7	24.9 ± 7.8	5	29.6 ± 14.1
	P	8	26.5 ± 14.7	6	30.0 ± 5.2	3	30.0 ± 17.7
Creatinine (mg/dL)	S	9	1.33 ± 0.44	7	1.29 ± 0.32	6	1.37 ± 0.29
	P	8	1.36 ± 0.34	6	1.38 ± 0.26	3	1.03 ± 0.49
ALT (U/L)	S	9	75.7 ± 54.7	7	98.3 ± 134	2	35.5 ± 0.7
	P	8	112 ± 129	6	93.5 ± 112	2	34.5 ± 20.5
Sodium (mmol/l)	S	9	147 ± 3.7	7	150 ± 5.0	5	142 ± 3.9
	P	8	148 ± 4.0	6	145 ± 4.4	3	147 ± 7.1
Potassium (mmol/l)	S	9	4.4 ± 0.6	7	4.1 ± 0.5	6	3.9 ± 0.5
	P	8	3.8 ± 0.3	6	3.7 ± 0.4	3	4.2 ± 0.5
Neuroendocrine markers							
NT-proANP (fmol/l) ^{(a),(b)}	S	9	0.70 ± 0.41	7	0.83 ± 0.55	5	0.83 ± 0.44
	P	7	0.95 ± 0.51	6	0.47 ± 0.46	2	0.32 ± 0.29
Aldosterone (pmol/l)	S	9	147 ± 144	7	96.5 ± 62.3	2	104 ± 32.2
	P	7	129 ± 76.3	5	172 ± 144	1	216

Parameters with significant time evolution are indicated with (a), parameters showing significantly different evolution between the two treatment groups are indicated with (b); Heart Failure Stage according to Scandinavian modified New York Heart Association System for heart failure classification in dogs with mitral valve disease (Table 1); S, spironolactone; P, placebo; PU/PD, polyuria and polydipsia; VHS, vertebral heart size; ALT, alanine aminotransferase; NT-proANP, aminoterminal atrial natriuretic propeptide.

Parameter	Group	n	Baseline	n	3 months	n	6 months
IVSd (mm)	S	8	8.7 ± 2.9	7	9.1 ± 3.5	5	11.3 ± 3.7
	P	9	8.9 ± 2.9	7	8.5 ± 4.0	6	6.8 ± 2.3
IVSs (mm)	S	8	13.8 ± 3.5	7	13.8 ± 4.5	5	14.4 ± 5.1
	P	9	12.3 ± 3.0	7	12.6 ± 3.6	6	12.7 ± 1.9
LVd (mm)	S	8	52.3 ± 20.1	7	49.1 ± 19.6	5	47.1 ± 19.5
	P	9	49.3 ± 17.2	7	48.9 ± 18.6	6	52.5 ± 21.0
LVs (mm)	S	8	35.8 ± 17.4	7	31.8 ± 16.5	5	34.1 ± 17.1
	P	9	36.0 ± 20.7	7	35.8 ± 22.2	6	35.6 ± 24.2
PWd (mm)	S	8	9.1 ± 2.7	7	10.7 ± 5.2	5	10.2 ± 3.1
	P	9	8.1 ± 2.0	7	8.4 ± 2.6	6	7.6 ± 1.9
PWs (mm)	S	8	13.4 ± 3.6	7	14.8 ± 4.8	5	14.9 ± 4.2
	P	9	10.6 ± 2.1	7	9.3 ± 5.1	6	12.3 ± 2.9
FS (%)	S	8	37.1 ± 12.9	7	37.9 ± 11.9	5	29.0 ± 13.4
	P	9	31.3 ± 16.2	7	31.7 ± 19.6	6	34.0 ± 15.9
EPSS (mm)	S	8	6.7 ± 6.4	7	6.2 ± 6.0	4	3.6 ± 2.7
	P	8	6.7 ± 7.3	7	2.1 ± 1.6	4	5.9 ± 8.1
LA2D (mm)	S	7	40.9 ± 24.9	6	48.4 ± 23.6	5	41.6 ± 24.1
	P	9	43.8 ± 10.3	5	50.3 ± 3.8	3	49.5 ± 11.7
Ao/LA	S	7	1.65 ± 0.84	6	2.00 ± 0.83	5	2.19 ± 0.50
	P	6	2.37 ± 0.90	5	2.51 ± 0.47	2	1.70 ± 1.13
Ao (m/s) ^(a)	S	6	0.81 ± 0.37	6	1.09 ± 0.31	5	1.04 ± 0.43
	P	9	1.34 ± 0.62	6	1.04 ± 0.21	4	1.19 ± 0.10
PA (m/s)	S	8	0.66 ± 0.09	7	0.68 ± 0.22	5	0.67 ± 0.17
	P	8	0.68 ± 0.18	7	0.69 ± 0.28	2	0.94 ± 0.12
PEP (ms)	S	6	65.5 ± 17.9	6	55.8 ± 25.5	5	55.7 ± 17.9
	P	8	68.9 ± 23.9	5	58.2 ± 12.5	3	60.7 ± 31.0
ET (ms)	S	6	155 ± 17.4	6	156 ± 26.8	5	156 ± 29.6
	P	8	162 ± 29.8	5	149 ± 32.3	3	148 ± 32.9
PEP/ET	S	6	0.43 ± 0.14	6	0.38 ± 0.21	5	0.37 ± 0.12
	P	8	0.44 ± 0.18	5	0.41 ± 0.14	3	0.39 ± 0.18
MV E/A	S	4	2.45 ± 1.17	3	2.33 ± 0.67	3	1.30 ± 0.26
	P	6	2.28 ± 1.35	6	1.72 ± 0.54	3	1.68 ± 0.57
EVEDt (m/s)	S	5	45.3 ± 20.6	3	36.2 ± 5.5	3	31.0 ± 7.2
	P	6	47.9 ± 34.0	5	40.4 ± 17.8	0	–

^(a)Significant time evolution; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVd, left ventricular diameter in diastole; LVs, left ventricular diameter in systole; PWd, posterior wall in diastole; PWs, posterior wall in systole; FS, fractional shortening; EPSS, mitral E-point septal separation; LA 2D, left atrial size before the opening of the mitral valve in the right parasternal long-axis view; Ao/LA, internal aortic diameter (Ao) and left atrial diameter from the right parasternal short-axis view; Ao, maximal aortic outflow velocity; PA, maximal pulmonic outflow velocity; PEP, pre-ejection period; ET, ejection time; MV E/A, mitral inflow E/A ratio; MV Edt, mitral inflow deceleration time of the E-wave.

DISCUSSION

The aim of this randomized prospective double-blinded placebo-controlled clinical study was to assess the efficacy and tolerance of spironolactone when added at a low dose to conventional heart failure treatment in dogs with advanced heart failure due to either DVD or DCM.

The results of this study show that addition of spironolactone at a dose between 0.49 and 0.8 mg/kg per day to conventional heart failure treatment was not associated with any adverse events. In particular, there was no measurable effect on plasma potassium concentrations. This finding is of importance, given reports of life-threatening hyperkalaemia in human patients treated with a combination of ACE inhibitors and spironolactone (Berry & McMurray, 2001; Juurlink *et al.*, 2004). The present

results are in accordance with the findings of Thomason *et al.* (2007) and Bernay *et al.* (2010), who reported no significant difference in serum potassium in dogs with DVD treated with a combination of ACE inhibitors and spironolactone at a mean dose of 1.49 ± 0.54 mg/kg twice daily and 2.33 ± 0.34 mg/kg once daily, respectively.

In this present study, no significant effect of spironolactone on survival could be shown. Dogs in the control group had a higher risk of moving into a higher heart failure stage than dogs in the spironolactone group during the 6 months of the trial. This might indicate a potential beneficial effect of spironolactone on the progression of heart failure, but this finding was not supported by other parameters evaluated in this study such as clinical score, ECG abnormalities, blood pressure, VHS and haemodynamic parameters.

Table 5. Haemodynamic parameters over the 6-month study period in the two treatment groups

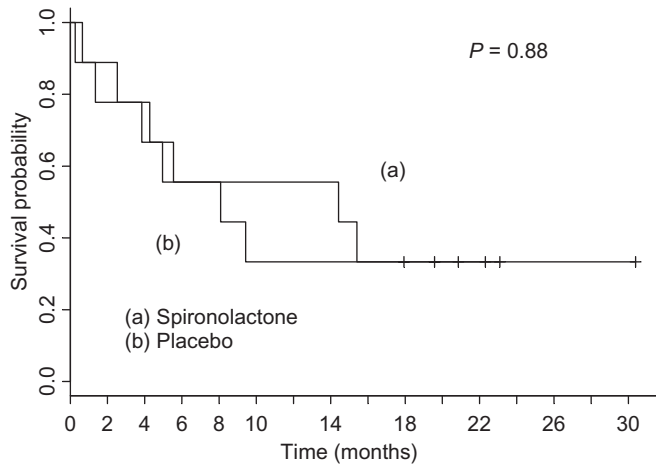


Fig. 1. Kaplan–Meier Curve showing lack of survival benefit in dogs with advanced degenerative valve disease or dilated cardiomyopathy treated with low-dose spironolactone in addition to conventional congestive heart failure treatment.

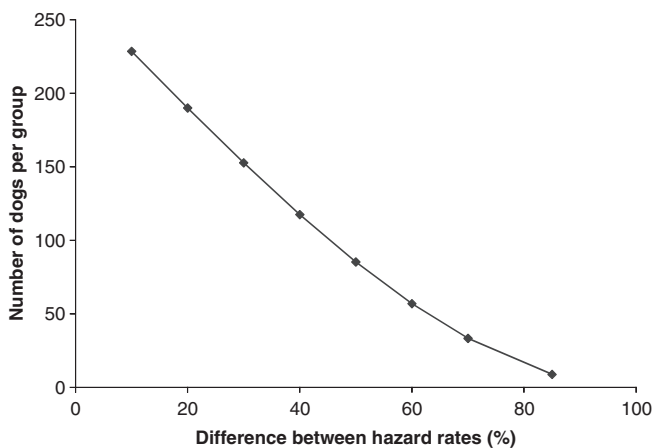


Fig. 2. Power calculation showing the minimum number of dogs per group needed to show a difference with an 80% statistical power over a 24-month period between the treatment and control groups. The hazard rate is the number of instantaneous deaths. According to the calculation, a minimum of 33 dogs per group are needed to show a significant difference between groups with a survival difference between groups of 70%.

The findings of the present study differ from human data from the RALES (Pitt *et al.*, 1999) and the study performed by Bernay *et al.* (2010) in a population of dogs with moderate to severe mitral valve disease. In the latter study, which was a prospective, placebo-controlled field trial including 212 dogs, a 69% reduction in risk of cardiac death or euthanasia was reported for the spironolactone group.

The exact mechanism of the potential beneficial effects of spironolactone in patients with heart failure is uncertain and may be multifactorial. There is strong evidence from humans and various animal heart failure models that mineralocorticoid receptor antagonists, such as spironolactone, reduce excessive

extracellular matrix turnover and myocardial remodelling, thus slowing down progression of heart disease (Rousseau *et al.*, 2002; Suzuki *et al.*, 2002; Hayashi *et al.*, 2003; Karram *et al.*, 2005). Study of a subgroup of the RALES patient population showed a significant decrease in BNP and NT-proANP associated with an increase in angiotensin II and aldosterone in patients treated with low-dose spironolactone, indicating effective aldosterone receptor blockade (Rousseau *et al.*, 2002). These findings provide further evidence for a potential neuroendocrine modulation as mechanism of action for the beneficial effects of spironolactone in heart failure patients (Rousseau *et al.*, 2002).

The lack of a measurable beneficial effect of spironolactone in this population of dogs could be a result of several factors including a small patient population, insufficient treatment dose and differences in baseline characteristics between spironolactone and control group.

The small patient number is a major limitation of this study. Power calculation performed after completion of the study showed that the number of dogs included was insufficient to conclusively demonstrate or disprove a survival benefit. Based on this data, up to 200 dogs per treatment group may be required to be able to show a significant difference between treatment groups. Such large patient numbers can only be completed in reasonable time using a multicentre approach, which have their own limitations, such as increased interobserver variability.

The low dose of spironolactone used may be another possible explanation for the lack of a detectable clinical effect of spironolactone in this study. This study was modelled after the RALES trial in which a low dose of 25 mg/person was used in an effort to avoid diuresis (Pitt *et al.*, 1999). We aimed at using a dose, which would be clearly below the approved diuretic dose in dogs (2–4 mg/kg once daily), to be able to show that potential positive effects of spironolactone are in fact due to other than diuretic effects. Specific pharmacological data from dogs were not available at the time of the study, thus the dose of 0.49–0.8 mg/kg once daily was chosen. In the meantime, data from a canine hyperaldosterone model, which was specifically designed to determine an effective dose of spironolactone for the management of congestive heart failure in dogs, has been published. In this experimental model a spironolactone dose of 1.08 ± 0.28 mg/kg only inhibited aldosterone actions by 50%. (Guyonnet *et al.*, 2009). Based on these results, it is likely that the dose used in the present trial was too low to provide sufficient aldosterone receptor blockade. This hypothesis is supported by the fact that plasma aldosterone did not increase in dogs in the spironolactone group during treatment which is in contrast to humans in the RALES study in which plasma aldosterone concentrations increased during treatment indicating activated feedback mechanisms (Rousseau *et al.*, 2002). A lack of effective aldosterone receptor blockage could explain the differences in outcome between the present study and the study of Bernay *et al.* (2010), in which a mean daily dose of spironolactone of 2.33 ± 0.34 mg/kg was used. Based on these findings, the use of a dose of 2 mg/kg once daily is advocated for further trials and active metabolites of spironolactone as well as plasma aldosterone should be measured to ascertain effective aldosterone

receptor blockade as well as owner compliance. These findings also emphasize the importance of dose-finding trials ahead of the study of specific clinical outcomes in relation to drug therapy.

There were some differences between the two treatment groups at baseline, which could account for the lack of measurable beneficial effect of spironolactone. Three dogs in the spironolactone group but only one dog in the control group showed atrial fibrillation, which may suggest more advanced disease and a potentially poorer prognosis in the dogs in this group (Calvert *et al.*, 1997). Secondly, there was a higher overall clinical score in the spironolactone group at baseline. When this difference was detected, the clinical score was introduced as a covariant into the statistical model to exclude bias because of existing baseline differences. However, even after adjustment for the difference in the baseline clinical scores, no significant effect of spironolactone could be found. On the other hand, dogs with DCM and DVD were equally distributed between the two treatment groups and the mean daily dose of furosemide, which can be used as an indicator of severity of cardiac disease, was not significantly different between treatment groups (Smith *et al.*, 2005).

Other findings of this study were, firstly, that dogs with DVD were significantly more likely to be presenting cough, which could be secondary to a relatively larger left atrial size because of more marked mitral regurgitation, leading to compression of the left mainstem bronchus. Secondly, it was found that the total daily dose of furosemide was inversely correlated with survival, which underlines the value of this parameter as a marker of severity of heart failure (Smith *et al.*, 2005). The results of this study showed no effect of pimobendan on survival; however, this was not an endpoint of the study.

Plasma aldosterone and NT-proANP were assessed as neuro-endocrine markers of heart failure. The results have to be interpreted with caution given that only a limited number of samples could be assessed, especially at 6 months. Plasma aldosterone was unaffected by spironolactone treatment. NT-proANP increased significantly over time in the spironolactone group and significantly decreased in the control group. This finding was unexpected. Human patients with hyperaldosteronism typically show high plasma ANP concentrations, which acts to counterbalance the aldosterone-induced sodium and water retention (Levin *et al.*, 1998). Therefore, with aldosterone receptor blockade via spironolactone, a decrease rather than an increase in NT-proANP had been expected. However, increases in NT-proANP may explain the lack of a survival benefit in the spironolactone group. NT-proANP has been associated with substantially decreased survival times in humans with congestive heart failure (Dickstein *et al.*, 1998). It was highly correlated with heart rate, echocardiographic dimensions of the left atrium and left ventricle and fractional shortening in dogs with DCM as well as enlargement of the left atrium and ventricle in dogs with chronic valvular disease and could therefore indicate that dogs in the spironolactone group had potentially more advanced heart disease (Häggström *et al.*, 1994; Tidholm *et al.*, 2001).

This study is of relevance for future trials. Firstly, the study design proved to be feasible in the setting of a busy teaching hospital. Secondly, power calculation indicated that at least 66 patients need to be included in the study to be able to show significant survival differences between treatment groups assuming a reduction in survival of 69%, as described by Bernay *et al.*, 2010. Therefore, a multicentre approach should be applied. Thirdly, a higher dose of spironolactone should be used, and active metabolites in plasma should be monitored to prove that an effective dose is used and to verify owner compliance.

CONCLUSIONS

In conclusion, it can be stated that addition of spironolactone at a low dose to conventional heart failure treatment (furosemide, ACE inhibitor, pimobendan, digoxin) in dogs with acquired heart disease is well tolerated. It may improve heart failure class but beneficial effects on survival could not be shown in this study.

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