

1 **The SEISICAT study: a pilot study assessing efficacy and safety of**
2 **spironolactone in cats with congestive heart failure secondary to**
3 **cardiomyopathy**

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15 Running Head: Spironolactone use in cats with cardiac failure

16

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23

24 **Abstract**

25 **Introduction**

26 The pathophysiology of heart failure involves activation of several neurohormonal
27 systems including the renin-angiotensin-aldosterone system. The mineralocorticoid
28 receptor antagonist spironolactone has been shown to be beneficial in humans and
29 dogs with heart failure.

30 The objective of this pilot study was to investigate the efficacy and safety of
31 spironolactone in cats with heart failure secondary to cardiomyopathy already treated
32 with furosemide and an angiotensin converting enzyme inhibitor.

33 **Animals**

34 Twenty cats with heart failure due to cardiomyopathy.

35 **Methods**

36 The study was a double blind, randomised, placebo-controlled, multicentre clinical
37 study assessing the effect of spironolactone on survival and clinical parameters in cats
38 with heart failure due to cardiomyopathy. The primary endpoint was mortality, defined
39 as death (spontaneous or by euthanasia) due to cardiac causes.

40 **Results**

41 Twenty cats were enrolled: 9 in the spironolactone group and 11 in the placebo group
42 of which 56% (5/9) and 0% (0/11) completed the 15-month period respectively. At
43 inclusion, differences in systemic blood pressure, body condition score,
44 electrocardiographic abnormalities and LA/Ao ratio suggested disease may be less
45 severe in the spironolactone group. Twenty-two percent (2/9) of cats in the
46 spironolactone group and 82% (9/11) in the control group reached the primary
47 endpoint (Fisher's exact test, $p = 0.0216$). No safety issues were identified in either

48 group.

49 **Conclusions**

50 This study suggests that spironolactone is well-tolerated and preliminary results
51 support further investigation to evaluate the efficacy of spironolactone in the treatment
52 of cats with cardiac failure due to cardiomyopathy.

53

54 **Key words:** aldosterone; feline; mineralocorticoid receptor

55

56 **Abbreviations**

ACEi	angiotensin converting enzyme inhibitor
Ao	aorta
CI	confidence interval
HCM	hypertrophic cardiomyopathy
HR	hazard ratio
LA	left atrium
RAAS	renin angiotensin aldosterone system
SD	standard deviation

57

58 **Introduction**

59 Cardiomyopathy is the most common form of heart disease and cause of heart failure
60 in cats [1]. The pathophysiology of heart failure involves activation of several
61 neurohormonal systems such as the sympathetic nervous system and the renin-
62 angiotensin-aldosterone system (RAAS) to compensate for the decrease in cardiac
63 output. Aldosterone is a steroid hormone with mineralocorticoid activity and its major
64 physiological function is maintaining sodium and potassium balance and blood
65 pressure control. Binding of aldosterone to the mineralocorticoid receptors in the
66 kidneys results in an increase in sodium and water reabsorption and potassium
67 secretion [2]. This phenomenon increases the extra-cellular fluid volume and thus
68 cardiac preload [3], helping maintain cardiac output in heart failure. Mineralocorticoid
69 receptor antagonists counteract the retention of sodium and water, and reduce
70 aldosterone-induced potassium loss [3, 4]. Mineralocorticoid receptors are also found
71 in cardiomyocytes, coronary endothelial and vascular smooth muscle cells, fibroblasts,
72 and inflammatory cells, such as macrophages [3]. Chronic activation of the RAAS is
73 thought to give rise to deleterious effects and worsening of cardiac function,
74 proarrhythmogenic effects, progression of myocardial fibrosis, vascular remodelling
75 and endothelial dysfunction [5]. Cardiac fibrosis has been reported in 53% of cases of
76 feline hypertrophic cardiomyopathy (HCM) [6] and in the myocardial type of feline
77 restrictive cardiomyopathy [6, 7, 8]. Mineralocorticoid receptor antagonists like
78 spironolactone have been shown to inhibit aldosterone-induced myocardial fibrosis [5]
79 and to reduce remodelling of the vascular smooth muscle cells and myocytes [3]. This
80 action may therefore represent an additional benefit to using these agents in feline
81 cardiomyopathy.

82 Aldosterone receptor blockade has now been shown to be beneficial in humans with
83 heart failure [9, 10, 11] and in dogs with heart failure secondary to mitral valve disease
84 [4]. Although there is little published work regarding the treatment of cats in heart
85 failure, the current recommendations for treatment include the use of diuretics,
86 angiotensin-converting enzyme inhibitors and antiplatelet drugs if antithrombotic
87 prophylaxis is required [12, 13]. One study of spironolactone at 2mg/kg per os twice
88 daily for 4 months in Maine Coon cats affected by HCM conducted by McDonald et al.
89 in 2008 did not show any improvement in the mitral annular velocity or reduction of the
90 left ventricular mass [14], four of the 13 treated cats developed severe ulcerative facial
91 dermatitis. However, this was a short study of only a few months in cats with sub-
92 clinical disease in a related population. The efficacy and safety of spironolactone are
93 poorly documented in cats with naturally occurring heart disease and congestive heart
94 failure and warrant further investigation due to the drug's mode of action, its antifibrotic
95 properties and its efficacy in heart failure in other species.

96 The objective of this pilot study was to assess the safety and efficacy of spironolactone
97 in cats with congestive heart failure secondary to cardiomyopathy being treated in
98 combination with furosemide and an angiotensin converting enzyme inhibitor (ACEi).

99 **Animals, Materials and Methods**

100

101 ***Study design***

102 The study protocol was approved by the Ethics Committee of the University of
103 Nottingham (reference 135 100118) and an Animal Test Certificate was obtained from
104 the UK Veterinary Medicines Directorate permitting the use of spironolactone in a
105 species for which it was not licensed. This pilot study was a double blind, randomised,
106 placebo-controlled, and multicentre clinical study.

107

108 ***Animals***

109 Client-owned cats with suspected heart failure were screened and those diagnosed
110 with heart failure fulfilling the inclusion criteria were asked to participate in the trial at
111 3 different cardiology speciality referral centres in the United Kingdom. Cases were
112 seen in practice as primary care or referred cases.

113

114 ***Inclusion criteria***

115 Cats were eligible for inclusion provided the owner had completed and signed an
116 informed consent form. To be enrolled in the study, cats, of any age, gender and breed,
117 had to present with congestive heart failure secondary to cardiomyopathy, with
118 presence of appropriate clinical signs and radiographic evidence of pulmonary
119 oedema and/or pleural effusion due to left sided or biventricular congestive heart
120 failure. The cardiomyopathy had to be confirmed by echocardiographic examination
121 by diploma-holding veterinary cardiologists using currently accepted diagnostic criteria
122 [8] to identify left ventricular remodelling, left atrial (LA) enlargement from both the

123 long (>16.5 mm) and the short axis views with a left atrial (LA)/Aorta (Ao) ratio of >
124 1.6, and Doppler evidence of diastolic dysfunction.

125

126 ***Exclusion criteria***

127 Cats with hyperthyroidism, hypertension (Doppler systolic blood pressure >180
128 mmHg), severe renal disease (serum creatinine >250µmol/l or 2.83mg/dl), congenital
129 heart disease, non-cardiac systemic disease which may affect the outcome (e.g.
130 thromboembolic disease or respiratory disease), pregnancy or lactation were
131 excluded. Cats were also ineligible if a dysrhythmia requiring the use of anti-arrhythmic
132 medication was evident or in cases of previous or ongoing treatment with pimobendan,
133 spironolactone or digoxin.

134

135 ***Randomisation***

136 Cats were randomly allocated to two treatment groups. A randomisation list was
137 prepared using 2N Software^c. Case allocation was stratified according to the presence
138 of hypertrophic cardiomyopathy or not, and then on the need for hospitalisation or not.
139 This led to the creation of four groups each with a respective randomisation list
140 composed of blocks of four.

141

142 ***Study drugs***

143 The animals were randomly allocated to two groups, the treated group received
144 spironolactone tablets^d and the control group received a placebo identical to
145 spironolactone in appearance and packaging. Cats were administered from half a
146 tablet to two tablets per day, *i.e.* a spironolactone dose between 1.72mg/kg and

147 3.33mg/kg per day. In cats, spironolactone is generally used at 2 to 4 mg/kg/day [12,
148 13]. An allometric extrapolation performed to identify a dose for use in cats provided a
149 similar theoretical dose of 3.15 mg/kg/day for cats with a mean body weight of
150 3.75kg. For ease of dosing and administration by the owners, only complete 10mg
151 tablets or half tablets were administered, resulting in the above mentioned dose (i.e.
152 1.72mg/kg to 3.33mg/kg per day). Study drugs were administered for sixty weeks,
153 once daily, with food. The tablet could either be mixed with a small amount of food
154 offered prior to the main meal or administered directly by mouth after feeding. The
155 owners were instructed to mark the date and time the medication was given on the
156 tablet packaging to aid with determination of compliance.

157

158 ***Concomitant treatments***

159 The cats of both groups had to be concomitantly treated with a combination of
160 furosemide and ACEi for congestive heart failure (at least from the day of inclusion).
161 Clopidogrel administration was permitted and left to investigator's discretion. However,
162 administration of pimobendan, anti-arrhythmic medication such as diltiazem, digoxin,
163 lidocaine or beta blockers was forbidden as was the administration of aspirin. Cats
164 previously or currently treated with spironolactone were also excluded from the study.
165 Other concomitant medications, therapies or vaccines were allowed as long as
166 investigators judged they did not interfere with the evaluation of the tested product.

167

168 ***Visit schedule***

169 Visits and assessments were carried out in accordance with the schedule detailed in
170 supplemental Table A on-line.

171

172 ***Parameter assessment***

173 The clinical parameters recorded were appetite change, dyspnoea, demeanour
174 change, behaviour change, syncope, ascites and signs of gastro-intestinal disease.

175 Body condition score was assessed for each cat on a five-point scale.

176 The radiographic parameters evaluated on plain lateral and dorso-ventral thoracic
177 radiographs were the Buchanan vertebral heart score [15], presence or absence of
178 pleural effusion, pulmonary oedema assessed as none, mild or moderate interstitial,
179 localised or generalised alveolar pattern and the presence or absence of pulmonary
180 venous congestion.

181 Systolic blood pressure was determined non-invasively at each visit, using a Doppler
182 probe and an appropriate sized inflatable cuff according to guidelines established by
183 the International Society of Feline Medicine^e.

184 The electrocardiographic parameters evaluated were heart rate and rhythm,
185 ventricular and supraventricular arrhythmias (presence or absence) and any other
186 abnormality using limb leads in right lateral or sternal recumbency.

187 The following echocardiographic parameters were evaluated on two-dimensional and
188 M-mode images: intraventricular septum thickness in diastole and left ventricular free
189 wall thickness in diastole from the right parasternal long axis view and left ventricular
190 internal dimension in diastole and in systole, left ventricular shortening fraction, left
191 ventricular free wall thickness in diastole and in systole, interventricular septum
192 thickness in diastole and in systole were evaluated with M-mode from a right
193 parasternal short axis view at the level of the chordae tendinae. Left atrial diameter
194 was measured both from right parasternal short and long axis views. Left atrial short

195 axis and aortic diameter were used to determine Left Atrium/Aorta ratio (LA/Ao).
196 Presence or absence of systolic anterior motion of the mitral valve was also assessed
197 on two-dimensional and M-mode echocardiographic images. Assessment of mitral
198 and pulmonary venous inflow patterns and isovolumetric relaxation time using pulsed-
199 wave spectral Doppler echocardiography was used to assess left ventricular diastolic
200 function. All echoparameters were measured using a concurrent electrocardiogram
201 trace to assist with appropriate timings except where cats were non-compliant.

202 An overall assessment of the cat was also completed at each visit by both the
203 investigator and the owner to assess whether they felt the cat was very well, well, poor
204 or very poor.

205 Blood samples were collected at each visit. Biochemistry and haematological variables
206 were measured and urinalysis was performed.

207 Treatment compliance was assessed at each follow-up visit by counting the number
208 of used and unused tablets and checking the dates and times noted by the owner on
209 the tablet packets.

210

211 ***Safety assessment***

212 The adverse events were reported during the course of the study by the investigators.
213 An adverse event is defined as “any observation in animals that is unfavourable and
214 unintended, and occurs after the use of a veterinary product or investigational
215 veterinary product, whether or not considered to be product related. It is considered
216 serious if fatal, life-threatening or resulting in permanent and prolonged signs in the
217 treated animals”. The adverse events were coded and grouped by organ (System
218 Organ Class) according to the VeDDRA hierarchical structure, as defined by the

219 European Medicines Agency^f. Relationship to the study treatment was assessed by
220 the investigator at the time of the exam.

221

222 ***Outcome***

223 The primary endpoint was mortality, defined as death (spontaneous or by euthanasia)
224 due to cardiac causes. The cause of any spontaneous death was evaluated by the
225 investigator.

226 Secondary efficacy endpoints were morbidity-mortality, defined as the combined
227 incidence of death (spontaneous or by euthanasia) due to cardiac causes and
228 treatment failure as defined by the addition of a forbidden cardiac drug or premature
229 removal due to cardiac causes (e.g. thromboembolic disease).

230 The safety of spironolactone was assessed by describing the frequency and nature of
231 adverse events and the evolution of haematological, clinical biochemical and urine
232 parameters in both groups. The results of quantitative blood biochemistry variables
233 and quantitative haematological variables recorded at inclusion and at each visit was
234 compared between the two groups.

235

236 ***Statistical methods***

237 Few studies have prospectively evaluated survival in feline heart failure, consequently
238 sample size calculation was not feasible and for the purposes of this pilot study the
239 number of cats to be enrolled was set at 10 animals per group.

240 The results were analysed with Per Protocol and Intention to Treat populations for the
241 primary endpoint. For the Intention to Treat analysis, any cat having received the
242 tested product or the placebo would be included in the efficacy analysis. Only cats for

243 which the protocol was strictly respected would be included in the Per Protocol
244 analysis. The significance threshold was set at $p=0.05$. Analyses were run on
245 commercially available software⁹.

246 The two treatment groups were described and compared on individual criteria on day
247 0. For qualitative variables chi-square or Fisher's exact test was used according to
248 expected values obtained. In case of normality of the data distribution Student's *t* test
249 was used to compare all continuous variables between the two groups. The variance
250 equality was tested using a Folded F test, in case of unequal variance the
251 Satterthwaite adjustment was used. In case of non-normality, a non-parametric
252 Wilcoxon-Mann-Whitney test was used.

253 If the end of the follow up period for a case was not related to an endpoint or if the
254 follow up was ongoing at the time of statistical analysis, the cases were censored.
255 Survival curves were generated by the Kaplan-Meier method. Survival analysis was
256 performed using a log rank test to compare the survival of the two treatment groups.
257 The hazard ratio (HR) and its 95% confidence interval (95% CI) were determined
258 based on a univariate analysis using a Cox model. Bivariate Cox proportional hazard
259 analysis was also performed. The global percentages of morbidity-mortality or
260 mortality events at the end of follow-up were compared between groups using Fisher's
261 exact test. Statistical significance was declared at a two-sided p -value of ≤ 0.05 .

262 **Results**

263 Twenty cats were enrolled, 9 in the spironolactone group and 11 in the placebo group,
264 and followed up for a maximum duration of 15 months, unless death or treatment
265 failure occurred. Fifteen cats presented with HCM and 7 cats required hospitalisation.
266 The results of the randomisation is described below in Table 1.

267 In the spironolactone-treated group, 7 cats (77.8%), had HCM, 1 cat had dilated
268 cardiomyopathy and 1 cat had unclassified cardiomyopathy. In the placebo-treated
269 group 8 cats (72.7%) had HCM, 1 cat had dilated cardiomyopathy, 1 cat had restrictive
270 cardiomyopathy and 1 cat had arrhythmogenic right ventricular cardiomyopathy. There
271 was no significant difference between the two groups in the echocardiographic
272 diagnoses.

273 Among the 20 cats enrolled, no cat experienced a major deviation such as
274 administration of a forbidden treatment or an evident lack of compliance, therefore, the
275 Per Protocol population was identical to the Intent To Treat population and the safety
276 population and includes all enrolled cases.

277

278 ***Study population at recruitment***

279 The spironolactone and the placebo groups were compared on demographic, clinical,
280 thoracic radiographic, electrocardiographic, echocardiographic and biochemical
281 parameters at inclusion. Baseline characteristics for the two groups are described in
282 Table 2.

283

284 ***Treatment***

285 Cats in the spironolactone-group received a median dose of 2.83 mg/kg (range 2.08

286 – 3.36 mg/kg, inter-quartile range 0.695), doses administered to individual cats are
287 shown in supplemental Table B on-line. Before inclusion, 17 cats received furosemide
288 with a time averaged daily dose \pm standard deviation (SD) of 5.0 (\pm 2.9) mg/kg and a
289 mean duration before enrolment (\pm SD) of 11 (\pm 10) days. Of these, 13 received
290 concomitant benazepril with a time averaged daily dose (\pm SD) of 0.6 (\pm 0.4) mg/kg
291 for a mean period (\pm SD) of 22 (\pm 53) days.

292 When included in the study, the time averaged daily dose (\pm SD) of furosemide for the
293 cats in the spironolactone- and in the placebo-treated groups were respectively 3.0 (\pm
294 1.2) mg/kg and 4.8 (\pm 2.3) mg/kg. For benazepril administration, cats received
295 respectively in the spironolactone- and in the placebo-treated groups 0.4 (\pm 0.2) mg/kg
296 and 0.6 (\pm 0.4) mg/kg as a time averaged daily dose (\pm SD).
297 During the study, 8 cats received a potassium supplementation: 5 in the
298 spironolactone treated group and 3 in the placebo-treated group.

299

300 ***Primary endpoint***

301 In the spironolactone and control groups, respectively, 56% (5/9) and 0% (0/11) of
302 cats completed the 15-month period. With respect to mortality due to cardiac causes,
303 the estimated 15-month survival rate was 78% for the cats treated with spironolactone
304 and conventional therapy, and 0% for the cats in the control group (log rank test, $p =$
305 0.011) (Figure 1). The univariate analysis of treatment demonstrated that the
306 spironolactone treated cats had a significant risk reduction for reaching the primary
307 endpoint when compared with the placebo treated cats (HR=0.158; $p=0.0226$; 95%
308 CI=0.032–0.772).

309 In the spironolactone group, 22% (2/9) of cats reached the primary endpoint and 82%

310 (9/11) in the control group (Fisher's exact test, $p = 0.0216$). Causes of withdrawals not
311 related to cardiac death were worsening of heart failure and a need for forbidden
312 concomitant treatment in 2 of 11 cats (18%) receiving the placebo and death or
313 euthanasia for non-cardiac reasons in 2 of the 9 cats treated with spironolactone
314 (22%).

315 Because of the significant difference identified between the groups in left atrial size at
316 baseline, this parameter was assessed as a covariate in the Cox model. When atrial
317 size and treatment group were included in a bivariate model, neither had a statistically
318 significant effect on survival, respectively $HR=1.53$; $p=0.53$; $95\%CI=0.391-6.240$ and
319 $HR= 0.199$; $p=0.07$; $95\%CI=0.026-1.063$

320

321 ***Secondary criteria***

322 *Morbidity-mortality*

323 Survival analysis showed an estimated 15-month survival rate of 78% in the
324 spironolactone-treated group significantly different to 0% in the placebo-treated group
325 (Log Rank test, $p=0.0042$). The results of univariate analysis on treatment effects
326 demonstrated a significant risk reduction in the spironolactone treated group as well
327 ($HR=0.136$; $p=0.0119$; $95\% CI=0.029-0.644$).

328 There is a significant difference in terms of number of events between the two groups
329 ($p=0.0005$) with morbidity-mortality in the placebo group significantly greater than
330 morbidity-mortality in the spironolactone-treated group (100% versus 22.2%).

331

332 *All causes of mortality*

333 Survival analysis showed an estimated 15-month survival rate of 56% in the

334 spironolactone-treated group and 0% in the placebo-treated group, which is not
335 significantly different (Log Rank test, $p=0.05$). Similarly, the results of the univariate
336 analysis on treatment effects show no significant risk reduction in the spironolactone
337 treated group (HR=0.309; $p=0.0604$; 95% CI=0.091–1.053). No significant difference
338 between the two groups ($p=0.16$) with all-cause mortality in terms of number of events
339 (81.8% versus 44.4%) was demonstrated. Cats with HCM represented 75% of the
340 population. For this reason, a post-hoc survival analysis on the sub-population of cats
341 with HCM ($n=15$, 7 in the spironolactone treated group and 8 in the placebo treated
342 group) was performed and showed an estimated 15-months survival rate of 100% in
343 the spironolactone-treated group, significantly different from 0% in the placebo-treated
344 group (Log Rank test, $p=0.0005$). There is a significant difference between the two
345 groups ($p=0.0014$) with mortality due to cardiac causes in the placebo group
346 significantly greater than in the spironolactone-treated group (percentage of events at
347 the end of follow-up: 87.5% versus 0.0%). The univariate analysis of treatment
348 demonstrated that the spironolactone treated cats had a significant risk reduction for
349 reaching the primary endpoint when compared with the placebo treated cats
350 (HR=0.033; $p=0.0335$; 95% CI=0.001–0.765).

351

352 **Safety**

353 *Adverse events*

354 In total, 39 adverse events were recorded during the study, with 16 recorded in 7 of
355 the cats (78%) in the spironolactone-treated group and 23 recorded in 11 of the cats
356 (100%) in the placebo-treated group. No skin and appendage disorders were recorded
357 during the course of the study. The adverse events according to the system organ

358 class classification^f are available in supplemental Table C on-line; most of the events
359 were metabolism and nutrition disorders (mainly hypokalaemia), digestive tract
360 disorders (vomiting) and systemic disorders (loss of appetite, euthanasia or sudden
361 death). Cardiovascular and respiratory tract disorders occurred primarily in the
362 placebo-treated group and were usually a consequence of worsening heart failure.
363 In the spironolactone-treated group, the investigators assessed 44% of the adverse
364 events as having no relationship to the product and 56% as having a possible or not
365 assessable relationship. In the placebo-treated group, the percentages were
366 respectively 39% and 61%.

367

368 *Blood and urine parameters*

369 The results for the haematological variables recorded at inclusion and the follow-up
370 visits for the two groups are shown in Supplemental Table D on-line.

371 The results for the quantitative blood biochemistry variables recorded at inclusion for
372 the two groups are shown in Supplemental table E on-line, the mean \pm SD of the
373 results are given. There was a significant difference between the two groups for ALT
374 activity, albumin and potassium. ALT activity and potassium concentration were higher
375 and albumin concentration was lower in the placebo-treated group compared to the
376 spironolactone-treated group. The changes in serum creatinine, urea, potassium and
377 sodium concentrations in the two groups with time are shown in Figure 2.

378 No abnormalities were diagnosed as a result of urinalysis that required any specific
379 interventions or suggested an adverse drug effect on the urinary system.

380

381

382 **Blood pressure**

383 The mean systolic blood pressure recorded at each visit from the cats in the two
384 groups is shown in Supplemental Table F on-line. There was a statistically significant
385 difference between groups in the systolic blood pressure with that of the placebo-
386 treated group being significantly higher at the first visit ($p=0.034$).

387

388 **Discussion**

389 This study demonstrated that the addition of spironolactone to conventional cardiac
390 therapy is safe and appears to reduce the risk of cardiac morbidity and mortality in
391 cats with cardiac failure due to cardiomyopathy when compared with conventional
392 therapy alone (ACEi plus furosemide). Of the nine cats treated with spironolactone,
393 five completed the 15 months study compared to none of the 11 cats treated with
394 placebo. These findings are consistent with survival studies on dogs [4] and humans
395 [9, 10, 11] with heart failure, although in this study the primary disease,
396 cardiomyopathy is different from that in the studies on dogs and humans.

397 However, interpretation of these efficacy data needs to be undertaken with caution
398 due to the small group sizes involved in this study and to the fact that the
399 randomisation process, by chance, led to the inclusion of cats with more severe heart
400 disease (larger left atrial size) in the placebo treated group. The assessment of the
401 impact of left atrial size on the efficacy of spironolactone in this study suggests that
402 this parameter should be included as a stratification factor in future studies.

403 A previous study in Maine Coon cats with familial hypertrophic cardiomyopathy,
404 spironolactone at 2mg/kg *per os* twice daily for 4 months did not improve the mitral
405 annular velocity nor reduced the left ventricular mass. Four of the 13 cats developed

406 facial dermatitis severe enough to warrant cessation of treatment [14]. Consequently,
407 one of the goals of the present study was to gather safety data on long-term use of
408 spironolactone in a group of out-bred cats to determine whether this problem was as
409 common in the general population. No cases of facial dermatitis were seen in the 9
410 cats treated for up to 15 months in the present study. Furthermore, the prevalence of
411 adverse events reported in the spironolactone treated cats was similar to those
412 reported in the placebo cats suggesting spironolactone is safe to use in cats with
413 naturally occurring heart failure when treated with furosemide and benazepril.
414 Interestingly, hypokalaemia was reported both in the spironolactone-treated group (3
415 cats) and in the placebo-treated group (2 cats). One explanation for the number of
416 spironolactone-treated cats having hypokalaemia may be that the mean serum
417 potassium concentration was significantly lower in the spironolactone group at
418 baseline when compared to the placebo group. Although these conclusions about the
419 safety of spironolactone are based on a small group of cats exposed to the drug for a
420 long period of time, the data are reassuring and support the design of larger pivotal
421 clinical trials in the future.

422 Most of the cats in this study (15/20) were suffering with HCM. The results of the
423 survival analysis in this population may not therefore be generalizable to cats with
424 other forms of cardiomyopathy. In feline medicine, the classification of myocardial
425 diseases traditionally follows the World Health Organisation definitions [16] and
426 guidelines for making these diagnoses are reported in the literature [8, 17, 18]. In
427 human medicine as well as in veterinary medicine the utility of this traditional
428 classification is starting to be questioned as more information regarding the natural
429 history of these conditions becomes available^h [19]. There is increasing evidence that

430 human and feline patients may transit between types of disease as the condition
431 progresses. Different phenotypic expressions of the same condition may exist
432 (possibly related to genetic heterogeneity [20, 21, 22]) and patients with a similar
433 cardiac phenotype may have a very different clinical course^h [23]. However, the
434 encouraging preliminary results on the HCM population would suggest that only cats
435 with HCM might be enrolled for a future clinical trial, as discussed above, stratified
436 according to left atrial size.

437 The activation of the RAAS system as a consequence of a fall in cardiac output
438 typically occurs in patients with cardiomyopathy of all types as congestive cardiac
439 failure develops. Consequently there is a degree of commonality in the
440 pathophysiological consequences of the primary disease in patients with
441 cardiomyopathy [8] with increased circulating levels of both aldosterone (initially at
442 least) and angiotensin II demonstrable in patients with cardiac failure. It is likely
443 therefore, that once cardiac failure has developed a similar pathophysiological process
444 is involved in the development of cardiomyopathy and the RAAS has been activated
445 [24].

446 No significant diuretic effect of spironolactone has been demonstrated in dogs [25, 26]
447 and it may be that the observed clinical benefit from associated with spironolactone
448 use arises primarily from mineralocorticoid blockade effects beyond those of diuresis.
449 It has been shown in rats [27, 28, 29] and human patients [30, 31, 32] that aldosterone
450 induces myocardial and perivascular fibrosis and alters the endothelial function of
451 vessels. Studies performed in human patients with congestive heart failure showed
452 that these effects are counteracted by mineralocorticoid receptor antagonists [32, 33].
453 Cats with hypertrophic cardiomyopathy have been shown to have significant interstitial

454 fibrosis and fibrosis of the endocardium [6, 8]. In human medicine there has been a
455 direct correlation shown between severity of diastolic dysfunction and amount of
456 myocardial fibrosis as documented on magnetic resonance imaging scans [34]. It has
457 also been shown in cats that cats with moderate or severe diastolic dysfunction have
458 a poorer prognosis [35], it is likely that this effect, at least in part is due to myocardial
459 fibrosis. Mineralocorticoid receptor antagonists like spironolactone have been shown
460 to inhibit aldosterone-induced myocardial fibrosis [5] and to reduce remodelling of the
461 vascular smooth muscle cells and myocytes [3]. This action may therefore represent
462 an additional benefit to using these agents in feline cardiomyopathy where inhibition
463 of profibrotic and prohypertrophic neurohormones is a reasonable treatment goal in
464 cardiomyopathies and other cardiac diseases [36]. More recently it has been
465 suggested that the benefit of mineralocorticoid receptor antagonists now extends to
466 the early phases of myocardial damage [37] and to heart failure with preserved
467 ejection fraction, a situation which could describe many cats with cardiomyopathy [38].
468 Other recent work also suggests that the effects of angiotensin II and mineralocorticoid
469 receptor activation in the heart are additive [39]. This observation may be relevant to
470 the clinical use of ACEi and mineralocorticoid receptor antagonists in combination in
471 heart failure.

472 The study has a number of limitations, which are largely a consequence of the fact
473 that it was a small scale pilot study. No power calculation was done to establish sample
474 sizes and the number of patients in each group is small. There were differences, some
475 significant, between the treatment group and the placebo group in some of the
476 baseline variables. In particular, in the placebo group the body weight at inclusion was
477 lower than that of the cats in the treatment group and the body condition score of

478 placebo-treated cats was significantly lower than that of the cats in the treatment
479 group. Similarly, in the placebo group there was a significantly higher prevalence of
480 other abnormalities on the electrocardiogram at presentation. In addition, on
481 echocardiographic assessment at inclusion into the study there was a significant
482 difference between the two groups in the LA/Ao ratio. Any future study might include
483 stratification of cats at inclusion according to severity of heart failure as assessed by
484 left atrial size or biomarker levels [40]. Another limitation is the fact that no dose
485 determination study was conducted prior to this pilot study. However, in practice,
486 veterinarians have been using spironolactone for several years using a dose range of
487 2 to 4 mg/kg [12, 13] and the allometric approach used in this study provided a similar
488 dose range (1.72-3.33 mg/kg). The study does suggest that spironolactone is safe to
489 use in cats and provides data which would permit better case selection and
490 stratification and a power calculation to be done for a full clinical trial.

491

492 **Conclusion**

493 Spironolactone therapy over a 15-month period in cats with heart failure secondary to
494 cardiomyopathy was safe to use and demonstrated a potentially beneficial effect when
495 added to conventional therapy. This finding needs to be confirmed by a large scale
496 clinical trial, with stratification at inclusion according to parameters which have recently
497 been demonstrated to be linked to prognosis.

498 Conflict of Interest Declarations:

499 The study was funded by Ceva Sante Animale. Emilie Guillot, Catherine Garelli-Paar
500 and Vanessa Grassi are employees of Ceva Sante Animale. Rachel James and
501 Malcom Cobb have received funding from Ceva Sante Animale within the last 5 years
502 for some or all of the following activities: research, travel, speaking fees and
503 preparation of educational materials.

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669 Footnotes.

670

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685 **Figure captions**

686

687 **Figure 1. Kaplan-Meier survival curves, showing the number of patients**
688 **surviving within the populations treated with spironolactone and placebo at**
689 **different time points.**

690

691

692 **Figure 2. Serum creatinine, urea, potassium and sodium mean concentrations**
693 **for the treatment and placebo groups with time, showing changes in these**
694 **parameters over the course of the study.**

695

V = visit number

696

697

698

Table 1. Randomisation of the enrolled cases into the treatment and placebo groups according to both stratifications					
	Cats with hypertrophic cardiomyopathy		Cats with other type of cardiomyopathy		Total
Need for hospitalisation	<i>Spironolactone</i>	<i>Placebo</i>	<i>Spironolactone</i>	<i>Placebo</i>	
Yes	2	3	1	1	7
No	5	5	1	2	13
TOTAL	7	8	2	3	20

Table 2. Baseline characteristics of the cats in the treatment and placebo groups at enrolment (mean ± standard deviation or number and percentage)				
		Spironolactone	Placebo	p-value
Clinical parameters				
	Weight (kg)	4.2 ± 1.3 (9)	3.6 ± 0.9 (11)	0.22
	Age (years)	7.0 ± 4.9 (9)	10.3 ± 3.5 (11)	0.09
	Breed			
	- Domestic Short Hair	8	9	
	- Ragdoll	-	1	
	- Birman	-	1	
	- Siamese	1	-	
	Blood pressure (mm Hg)	115± 22	137 ± 20	0.034
	Body Condition Score	4.3 ± 1.0	3.3 ± 1.2	0.048
Electrocardiography				
	Heart rate	185± 28	185±38	0.97
	Normal sinus rhythm	8/9 (89%)	7/11 (64%)	0.32
	Ventricular premature complexes	4/9 (44%)	3/11 (27%)	0.64
	Ventricular tachycardia	1/9 (11%)	0/11 (0%)	0.45
	Supraventricular premature complexes	0/9 (0%)	2/11 (18%)	0.48
	Atrial fibrillation	0/9 (0%)	2/11 (18%)	0.48
	Other arrhythmia	0/9 (0%)	1/11 (9%)	1.00
	Other abnormality*	0/9 (0%)	7/11 (64%)	0.0047
Echocardiography				
	Interventricular septum thickness in diastole (mm)	5.6± 1.5 (9)	6.2±2.1(11)	0.48
	LV internal dimension in diastole (mm)	14.5± 4.0 (9)	15.1±2.4(11)	0.72

LV free wall thickness in diastole (mm)	6.0± 2.3 (9)	5.7±1.5 (11)	0.68
Interventricular septum thickness in systole (mm)	7.7± 1.7 (9)	7.7±2.1 (11)	0.97
LV internal dimension in systole (mm)	7.8± 3.6 (9)	9.9±3.4 (11)	0.20
LV free wall thickness in systole (mm)	8.2± 2.5 (9)	7.2±2.0 (11)	0.30
LV shortening fraction (mm)	45.0± 14.9 (9)	35.4±14.1 (11)	0.15
Left Atrium short axis (mm)	17.0± 2.6 (9)	20.0±4.1 (11)	0.077
Left Atrium diameter from right parasternal long axis view (mm)	18.5± 2.4 (9)	22.6±5.0 (11)	0.051
Aorta diameter (mm)	9.0± 0.8 (9)	8.0±0.6 (11)	0.006
Left atrium/Aorta	1.9± 0.3 (9)	2.5±0.6 (11)	0.013

*Right bundle branch block, negative QRS in lead I but positive in leads II and III, tall and wide P waves, right axis deviation, second degree atrioventricular block, ST elevation, left bundle branch block.

Supplemental Table B. Doses of spironolactone administered respectively to each cat in the spironolactone-group

Cat	<i>Spironolactone dose (mg/kg)</i>
Cat 01 - A	3.26
Cat 02 - A	2.08
Cat 03 - A	2.90
Cat 04 - A	2.33
Cat 05 - A	2.83
Cat 06 - A	2.63
Cat 07 - A	3.03
Cat 08 - A	2.44
Cat 09 - A	3.13

Supplemental Table C. Number of adverse events in the treatment and placebo groups.

System Organ Class	Number of events reported			Number of cats presenting with the event (at least once)		
	Total	Spironolactone	Placebo	Total	Spironolactone	Placebo
Cardio-vascular system	8	1	7	8	1	7
Digestive tract	3	3	0	2	2	0
Ear and labyrinth	1	1	0	1	1	0
Hepato-biliary	1	1	0	1	1	0
Metabolism and nutrition	8	6	2	5	3	2
Renal and urinary	1	0	1	1	0	1
Respiratory tract	9	1	8	5	1	4
Systemic	8	3	5	6	2	4
Total	39	16	23	18	7	11

Supplemental Table D. Range of haematological variables (number of cats) for the cats in the treatment and placebo groups at each visit

Time	Haematocrit (%)		Packed cell volume (l/L)		Total platelet count (X10 ¹² /L)		Total Red cell count (X10 ⁹ /L)		Total white cell count (X10 ⁹ /L)	
	Spironolactone	Placebo	Spironolactone	Placebo	Spironolactone	Placebo	Spironolactone	Placebo	Spironolactone	Placebo
V1	31.8 – 44.3 (5)	23.0 – 42.5 (9)	32.0 (1)	27.0 – 42.0 (2)	75 - 757 (5)	65 - 582 (9)	6.1 – 9.7 (5)	4.4 – 9.6 (9)	5.2 – 14.7 (5)	5.5 – 32.1 (9)
V5	12.8 – 39.4 (4)	22.9 – 39.3 (3)	30.0 (1)	36.0 (1)	131 - 1046 (4)	297 - 378 (3)	3.0 – 9.5 (4)	5.4 – 9.6 (3)	5.3 – 10.1 (4)	6.3 – 14.9 (3)
V8	29.7 – 34.4 (3)	(0)	28.0-32.0 (4)	(0)	219 - 703 (3)	(0)	7.1 – 9.2 (4)	(0)	4.4 – 18.6 (4)	(0)
V9	26.4 – 30.0 (2)	(0)	32.0 – 35.0 (2)	(0)	310 - 1073 (3)	(0)	5.7 – 7.0 (3)	(0)	3.4 – 11.9 (3)	(0)

Supplemental Table E. Comparison of the baseline clinical biochemistry parameters of the cats in the treatment and placebo groups at enrolment (mean ± SD)

		Spironolactone	Placebo	p-value
	Alkaline phosphatase (U/l)	31.7±14.1	44.2±34.4	0.41
	Alanine aminotransferase (U/l)	47.0±19.9	89.2±52.3	0.040
	Albumin (g/l)	32.7±1.6	29.3±3.5	0.044
	Amylase (U/l)	950±281	1086±304	0.43
	Chloride (U/l)	114±5	119±4	0.08
	Cholesterol (mmol/l)	3.9±1.1	4.8±1.2	0.16
	Creatinine (µmol/l)	154±28	189±56	0.15
	Globulin (g/l)	45.0±5.8	47.1±11.4	0.68
	Phosphate (mmol/l)	1.6±0.2	1.5±0.2	0.46
	Potassium (mmol/l)	3.3±0.2	4.1±0.7	0.028
	Sodium (mmol/l)	155±8	160±6	0.25
	Bilirubin (µmol/l)	5.8±4.0	5.6±1.5	0.87
	Calcium (mmol/l)	2.4±0.1	2.4±0.2	0.88
	Protein (g/l)	78±6	76±12	0.80
	Urea (mmol/l)	11.6±7.9	14.5±7.5	0.41

Supplemental Table F. Systolic blood pressure (median and range) in mmHg (number of cats) in the treatment and placebo groups at each visit (V: visit number)

	V1	V2	V3	V4	V5	V6	V7	V8	V9
Spironolactone	120 85 – 144 (9)	121 106 – 134 (8)	128 120-148 (7)	132 104 – 141 (7)	132 112 – 144 (7)	136 120 – 158 (5)	134 96 – 151 (6)	116 112 – 152 (5)	124 120-128 (5)
Placebo	140 100 – 168 (11)	150 108 – 166 (11)	129 108 – 200 (10)	138 124 – 176 (6)	142 118 – 180 (5)	142 108 – 148 (3)	112 110 – 114 (2)		

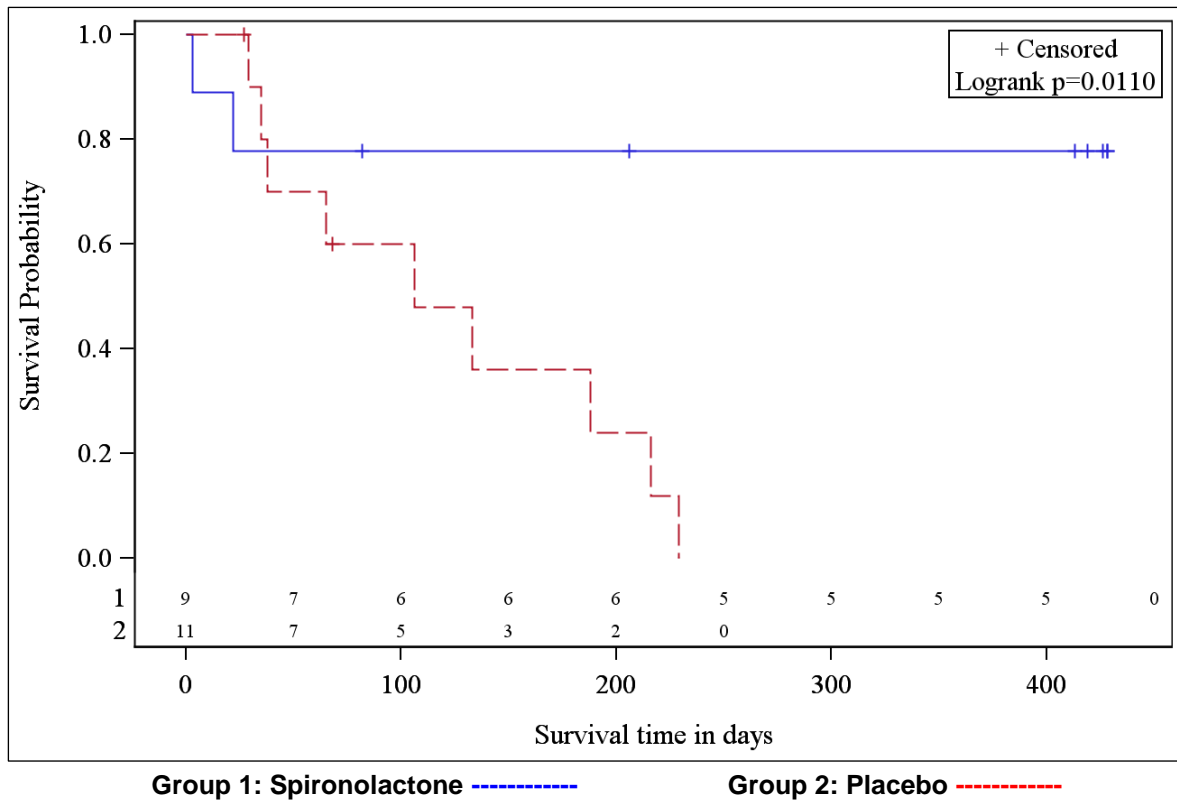


Figure 1

<p>Creatinine ($\mu\text{mol/l}$)</p>	<p>Legend: Spirolactone (blue line with diamonds), Placebo (orange line with squares)</p> <table border="1"> <thead> <tr> <th>Assessment time</th> <th>Spirolactone ($\mu\text{mol/l}$)</th> <th>Placebo ($\mu\text{mol/l}$)</th> </tr> </thead> <tbody> <tr><td>V1</td><td>150</td><td>185</td></tr> <tr><td>V2</td><td>190</td><td>175</td></tr> <tr><td>V3</td><td>165</td><td>165</td></tr> <tr><td>V4</td><td>160</td><td>195</td></tr> <tr><td>V5</td><td>185</td><td>160</td></tr> <tr><td>V6</td><td>180</td><td>185</td></tr> <tr><td>V7</td><td>155</td><td>240</td></tr> <tr><td>V8</td><td>160</td><td>160</td></tr> <tr><td>V9</td><td>180</td><td>160</td></tr> </tbody> </table>	Assessment time	Spirolactone ($\mu\text{mol/l}$)	Placebo ($\mu\text{mol/l}$)	V1	150	185	V2	190	175	V3	165	165	V4	160	195	V5	185	160	V6	180	185	V7	155	240	V8	160	160	V9	180	160
Assessment time	Spirolactone ($\mu\text{mol/l}$)	Placebo ($\mu\text{mol/l}$)																													
V1	150	185																													
V2	190	175																													
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V4	160	195																													
V5	185	160																													
V6	180	185																													
V7	155	240																													
V8	160	160																													
V9	180	160																													
<p>Urea (mmol/l)</p>	<p>Legend: Spirolactone (blue line with diamonds), Placebo (orange line with squares)</p> <table border="1"> <thead> <tr> <th>Assessment time</th> <th>Spirolactone (mmol/l)</th> <th>Placebo (mmol/l)</th> </tr> </thead> <tbody> <tr><td>V1</td><td>11.5</td><td>14.5</td></tr> <tr><td>V2</td><td>9.5</td><td>12.5</td></tr> <tr><td>V3</td><td>10.5</td><td>13.5</td></tr> <tr><td>V4</td><td>7.5</td><td>14.5</td></tr> <tr><td>V5</td><td>9.5</td><td>13.5</td></tr> <tr><td>V6</td><td>9.0</td><td>16.0</td></tr> <tr><td>V7</td><td>8.0</td><td>18.0</td></tr> <tr><td>V8</td><td>10.5</td><td>10.5</td></tr> <tr><td>V9</td><td>11.5</td><td>10.5</td></tr> </tbody> </table>	Assessment time	Spirolactone (mmol/l)	Placebo (mmol/l)	V1	11.5	14.5	V2	9.5	12.5	V3	10.5	13.5	V4	7.5	14.5	V5	9.5	13.5	V6	9.0	16.0	V7	8.0	18.0	V8	10.5	10.5	V9	11.5	10.5
Assessment time	Spirolactone (mmol/l)	Placebo (mmol/l)																													
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V8	10.5	10.5																													
V9	11.5	10.5																													
<p>Potassium (mmol/l)</p>	<p>Legend: Spirolactone (blue line with diamonds), Placebo (orange line with squares)</p> <table border="1"> <thead> <tr> <th>Assessment time</th> <th>Spirolactone (mmol/l)</th> <th>Placebo (mmol/l)</th> </tr> </thead> <tbody> <tr><td>V1</td><td>3.2</td><td>4.0</td></tr> <tr><td>V2</td><td>3.5</td><td>3.7</td></tr> <tr><td>V3</td><td>4.0</td><td>4.0</td></tr> <tr><td>V4</td><td>3.9</td><td>4.0</td></tr> <tr><td>V5</td><td>4.0</td><td>4.3</td></tr> <tr><td>V6</td><td>3.8</td><td>3.7</td></tr> <tr><td>V7</td><td>3.8</td><td>3.7</td></tr> <tr><td>V8</td><td>3.5</td><td>3.7</td></tr> <tr><td>V9</td><td>3.8</td><td>3.8</td></tr> </tbody> </table>	Assessment time	Spirolactone (mmol/l)	Placebo (mmol/l)	V1	3.2	4.0	V2	3.5	3.7	V3	4.0	4.0	V4	3.9	4.0	V5	4.0	4.3	V6	3.8	3.7	V7	3.8	3.7	V8	3.5	3.7	V9	3.8	3.8
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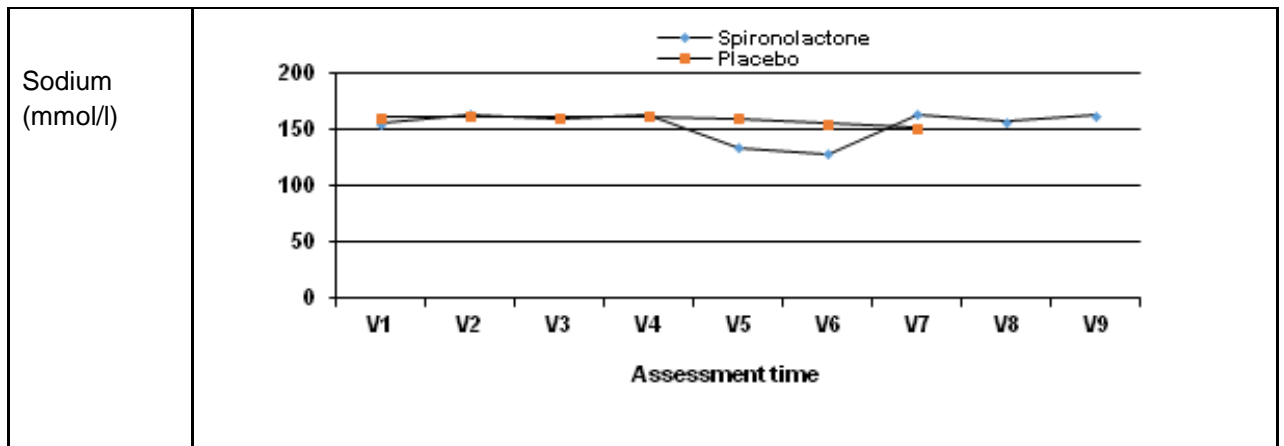


Figure 2