

# Evaluation of a fixed-dose combination of benazepril and pimobendan in dogs with congestive heart failure: a randomized non-inferiority clinical trial

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A fixed-dose combination tablet of benazepril and pimobendan (Fortekor Plus; Elanco Animal Health) was tested in dogs with congestive heart failure (CHF) caused by myxomatous mitral valve disease (MMVD) in a three-arm, masked, randomized, non-inferiority clinical trial in Japan. The test group (n = 34) received Fortekor Plus twice daily. Two control groups received registered formulations of benazepril (Fortekor; Elanco Animal Health) and pimobendan (Vetmedin; Boehringer Ingelheim Vetmedica) with administration of Vetmedin twice daily and Fortekor twice (Control I, n = 14) or once (Control II, n = 19) daily. Diuretics were used in 22 dogs (32.8%). Global clinical scores decreased significantly from baseline in all groups; there were no significant differences between groups, and non-inferiority of Fortekor Plus compared to Control I, Control II, and combined Control I + II groups was demonstrated. There were no significant differences between groups for relevant clinical chemistry and hematology variables or frequency of all adverse events. Frequency of emesis was significantly ( $p = 0.0042$ ) lower in the Fortekor Plus (8.8%) group than in the Control I + II (39.4%) group. In conclusion, Fortekor Plus had non-inferior efficacy and was associated with significantly less emesis compared to Fortekor and Vetmedin in dogs with CHF caused by MMVD.

**Keywords:** benazepril, congestive heart failure, dogs, pimobendan

## Introduction

Congestive heart failure (CHF) is a common clinical syndrome that results from a variety of disease processes in dogs, and a number of pharmaceuticals are used in the management of CHF cases. The standard treatment of CHF in dogs includes “triple therapy”, consisting of a combination of the diuretic furosemide, an angiotensin-converting enzyme (ACE) inhibitor (ACEI), and pimobendan [1]. Benazepril (Fortekor; Elanco Animal Health, USA) is an ACEI that reduces concentrations of angiotensin-2 and aldosterone [14,16], while pimobendan (Vetmedin; Boehringer Ingelheim Vetmedica, Japan) is an inodilator that produces a combination of vasodilation via inhibition of phosphodiesterase III and inotropy through calcium sensitization [6]. Benazepril and pimobendan, administered independently, have been shown to

improve clinical signs and increase survival time in dogs with CHF caused by valvular disease [3,11,15,18], and both drugs are registered in the European Union, Japan, and other countries for the treatment of CHF in dogs. Recently pimobendan was reported to prolong the preclinical period in dogs with myxomatous mitral valve disease (MMVD) [5].

A consequence of the use of triple therapy is the need to administer at least three medicines orally to dogs with CHF. In human medicine, compliance in patients taking their medicines as directed is inversely related to the complexity of the regimen and the number of doses prescribed per day [7]. Fixed-dose combination products have been shown to reduce the risk of non-compliance in humans, including those with cardiovascular disease, and a number of such products are available [2].

Elanco Animal Health (Greenfield, USA) developed fixed-dose combination tablets of benazepril and pimobendan

Received 1 Sep. 2016, Revised 1 Mar. 2017, Accepted 26 Mar. 2017

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pISSN 1229-845X

eISSN 1976-555X

(Fortekor Plus; Elanco Animal Health) on the basis of the drugs' complementary therapeutic actions and in order to increase treatment compliance and ease animal handling. Fortekor Plus is available in two tablet sizes, containing 2.5 or 10 mg of benazepril hydrochloride and 1.25 or 5 mg of pimobendan, respectively. Benazepril (Fortekor) is registered to be administered once daily (SID) in a dosage range of 0.25 to 1.0 mg/kg, allowing a starting dosage of 0.25 to 0.5 mg/kg to be doubled to 0.5 to 1.0 mg/kg if needed. Pimobendan (Vetmedin) is registered in Japan at a dosage of 0.25 mg/kg twice daily (BID) with a range of 0.17 to 0.35 mg/kg on the dosage chart. Pimobendan must be administered BID due to its short half-life [6]. Therefore, in the fixed-dose combination, benazepril is administered BID with no possible doubling of the dose.

The aim of the present study was to test the field efficacy and safety of Fortekor Plus tablets in dogs with CHF caused by MMVD and, specifically, to support the registration of Fortekor Plus in Japan. The first objective was to compare the effects of Fortekor Plus with those of a combination of Fortekor and Vetmedin. A second objective was to compare the effects of benazepril when dosed BID and SID. The study was a prospective, multi-center, parallel-group, three-arm, masked, randomized, non-inferiority design clinical trial.

## Materials and Methods

The study was conducted in compliance with Good Clinical Practice guidelines [Ministry of Agriculture, Forestry and Fisheries, Japan, Ordinance nos. 75 (1997) and 36 (amendment 2005)]. The study protocol was reviewed and approved by the

Elanco Animal Health Global Animal Welfare Officer (JPN-12-001). This manuscript was prepared after consulting the checklist in the extension of the CONSORT statement for reporting of non-inferiority trials [17].

The study schedule is shown in Table 1. Dogs were examined at day (D)0 and their case histories recorded. Animals were included in the study after confirmation of their eligibility and written owner consent had been obtained.

### Inclusion criteria

Dogs with CHF associated with MMVD were recruited. All of the following criteria had to be present at baseline: 1) characteristic murmur associated with mitral regurgitation; 2) cardiac enlargement on chest X-ray; 3) modified New York Heart Association (NYHA) Functional Classification Class III or IV heart failure [11]. Note that for NYHA Class III or IV, all dogs had to have clinical signs consistent with CHF and radiographic evidence of pulmonary edema; and 4) body weight  $\geq 2.5$  kg (to permit accurate dosing of the test articles).

### Exclusion criteria

Dogs meeting any of the following criteria were not eligible for enrollment: 1) clinically relevant heart disease other than acquired MMVD (*e.g.*, congenital heart disease). Note that dogs with tricuspid valve disease were also eligible provided that the primary cause of CHF was MMVD; 2) clinically relevant concurrent systemic disease judged likely to negatively affect the clinical status or survival of the dog in the next 3 months (*e.g.*, diabetes mellitus, neoplasia, kidney or liver disease). Pre-renal azotemia was not considered an exclusion

**Table 1.** Study schedule

Responsible		Event/day (D)	D0	D7 $\pm$ 1	D14 $\pm$ 2	D28 $\pm$ 2	D56 $\pm$ 3	At withdrawal
		Visit to clinic	X	X	X	X	X	X
Investigator	Case history and baseline		X					
	Inclusion/exclusion criteria		X					
	Owner's consent		X					
	Physical examination, bodyweight and BCS		X	X	X	X	X	X
	GCS		X	X	X	X	X	X
	Modified NYHA classification		X			X	X	X
	Owner assessment (FETCH)		X	X	X	X	X	X
	Thoracic radiography		X			X	X	X
	Withdrawal criteria		X	X	X	X	X	
	Clinical chemistry and hematology		X			X	X	X
AEs occurrence			X	X	X	X	X	
Dispenser	Allocation to group		X					
	Prescription/return and check of test articles		X	X	X	X	X	X

BCS, body condition score; GCS, global clinical score; NYHA, New York Heart Association; FETCH, functional evaluation of cardiac health; AEs, adverse events.

criterion; 3) clinically relevant gastrointestinal disease likely to affect the absorption of the test articles; 4) females that were pregnant, lactating, or intended for breeding; or 5) known sensitivity to benazepril or pimobendan.

### Randomization

The dogs were randomized to one of three groups (Table 2); investigational veterinary product (IVP), Control I, and Control II groups in a 2:1:1 ratio. Within groups, dogs were stratified according to previous treatment or no treatment with pimobendan. Separate randomization lists were generated for each site by a statistician. The block length for each stratum was 4 dogs.

### Test articles

The IVP consisted of flavored tablets containing, respectively, 2.5/1.25 mg or 10/5 mg of benazepril hydrochloride/pimobendan (Fortekor Plus). The control products (CPs) consisted of flavored tablets containing 2.5 or 5 mg of benazepril hydrochloride (Fortekor 2.5 and 5 mg) or 1.25 or 5 mg of pimobendan (Vetmedin).

The IVP group received benazepril (0.25–0.5 mg/kg BID) and pimobendan (0.125–0.25 mg/kg BID) at dosages registered for Fortekor Plus in Japan (Table 2). The Control II group received benazepril (starting dosage 0.25–0.5 mg/kg SID with the option to increase to 0.5–1.0 mg/kg SID if judged necessary by a veterinarian) and pimobendan (0.25 mg/kg BID) at the dosages registered in Japan. The Control I group received the same daily dosages as the Control II group; however, the benazepril was administered BID (0.125–0.25 mg/kg BID with the option to increase to 0.25–0.5 mg/kg BID).

All test articles were administered orally for  $56 \pm 3$  days starting on D0. To be in compliance with the Vetmedin label, the IVP and CPs were administered at least 1 h before providing any feed. Drugs administered BID were given approximately 12 h apart.

If a dog was already receiving an ACEI or pimobendan or

both at inclusion, then, for animal welfare reasons, those drugs were discontinued only on the day before starting treatment with IVP or CPs with no wash-out period.

### Blinding

The IVP and CPs have different appearances and packaging. Masking of the investigators who made the clinical evaluations was achieved via the use of a dispenser at each site who dispensed the test articles to the owners, checked the owner dosing forms, and performed the reconciliation of products dispensed to and returned by the owners. Owner assessments were not blinded.

### Concomitant treatments

Standard concomitant therapy for CHF (including furosemide, digoxin, spironolactone, and amlodipine) or treatments with no reported effect on the cardiovascular system (*e.g.*, antiparasite agents, vaccines) were permitted throughout the study. With the exception of the IVP or CPs, the following were prohibited: pimobendan, levosimendan or similar inotropes or inodilators, benazepril or any other ACEI, angiotensin II receptor antagonists, renin inhibitors, and xanthines.

### Follow-up

If enrolled into the study, the dogs were examined again at  $D7 \pm 1$ ,  $D14 \pm 2$ ,  $D28 \pm 2$  and  $D56 \pm 3$ , and at the time of any premature withdrawal. On each examination day, a physical examination was performed, and body weight, rectal temperature, body condition score (BCS), and signs of CHF were recorded.

Cases could be prematurely withdrawn from the study for various reasons including adverse events (AEs) that were serious or could interfere with the evaluation of the test articles, major protocol deviations, or worsening of signs of CHF. Treatment failure was defined as the occurrence of: death or euthanasia; one or more of persistent dyspnea, progressive ascites, severe cachexia, or marked exercise intolerance,

**Table 2.** Study groups

Formulation	IVP (n = 34)		Control I (n = 14)		Control II (n = 19)	
	Benazepril	Pimobendan	Benazepril	Pimobendan	Benazepril	Pimobendan
	Fortekor Plus	Fortekor Plus	Fortekor	Vetmedin	Fortekor	Vetmedin
Dosage range (mg/kg)	0.25–0.5 BID	0.125–0.25 BID	0.125–0.5 BID*	0.17–0.35 BID	0.25–1 SID*	0.17–0.35 BID
Mean (range) daily dosage (mg/kg)	0.72 (0.51–1.0)	0.36 (0.26–0.50)	0.50 (0.25–0.93)	0.49 (0.36–0.60)	0.54 (0.24–1.0)	0.48 (0.37–0.60)

IVP, investigational veterinary product; BID, twice daily; SID, once daily. \*In compliance with the Fortekor label in Japan, the starting daily dosage of benazepril (0.25–0.5 mg/kg/day) could be doubled to 0.5–1.0 mg/kg/day by a veterinarian. This was done in 8/14 dogs (57.1%) in Control I, and 9/19 (47.4%) of dogs in Control II.

attributed to a cardiac cause, despite receiving or failing to tolerate furosemide (up to a maximum of 12 mg/kg/day orally) plus spironolactone (up to a maximum of 6 mg/kg/day orally) in addition to the test articles; or the need for additional cardiac medications not permitted in the protocol. The cause and the relationship or not to heart failure of any death or euthanasia were recorded, and it was noted if death was sudden or a result of progressive deterioration in the dog's health.

### Efficacy assessment

Assessments were made at D0, D7 ± 1, D14 ± 2, D28 ± 2, and D56 ± 3, and at times of premature withdrawal.

The primary endpoint was the global clinical score (GCS), which was the unweighted sum of the numerical rating scale scores determined by the investigator for exercise tolerance (range, 1–4), demeanor (range, 1–4), appetite (range, 1–4), respiratory effort (range, 1–4), coughing (range, 1–4) and nocturnal dyspnea (range, 1–3) using the scoring schemes described by Häggström *et al.* [11]. The GCS ranged from 6 to 23, with a lower score indicating milder signs of CHF.

Secondary endpoints were the investigators' assessments of individual parameters of the GCS [11], the owners' assessment of functional evaluation of cardiac health (FETCH) score [10], and the proportion of dogs with an improvement in modified NYHA classification score [11]. In addition, right lateral and dorsoventral thoracic radiographs were taken at D0, D28 ± 2, and D56 ± 3, and assessments made of heart size and pulmonary edema using 1 to 4 scales. The pulmonary edema scoring scheme was 1 = none; 2 = mild interstitial opacity; 3 = moderate interstitial opacity; and 4 = alveolar pattern, severe consolidation [11].

### Safety assessment

Safety was assessed from the nature and occurrence of AEs. In addition, venous blood samples for routine hematology and clinical chemistry were taken on D0, D28 ± 2, and D56 ± 3 (and at the time of any premature withdrawal). Lastly, investigators examined the dogs at each visit and recorded general condition (appetite, vigor, and fecal consistency) and BCS using a 1 to 5 scale (cachectic, thin, optimal, overweight, or obese). If an arrhythmia was suspected on auscultation, an electrocardiogram was recorded.

### Statistics

Data are presented as mean ± SD values or a range unless otherwise stated. All statistical tests were performed by using SAS software (ver. 8.2; SAS Institute, USA).

Groups were compared at baseline by performing analysis of variance (ANOVA) as appropriate for normally distributed data, or by using Mann-Whitney, Kruskal-Wallis, or Fisher exact probability tests.

Efficacy, clinical chemistry, and hematology variables were analyzed by using repeated measures analysis of covariance

(RMANCOVA) with the following covariates: treatment; previous and concomitant treatment with ACEI, pimobendan, diuretic (spironolactone excluded), spironolactone or amlodipine; time in study (week), time × treatment interaction, and baseline. Since spironolactone and amlodipine treatments were in all cases continuations of previous therapy, use of each of these drugs was evaluated in a single category. Backward variable selection was employed so that if  $p > 0.1$  for any covariate, the covariate with the highest  $p$  value was removed from the model, which was repeated until  $p$  values for all remaining covariates were  $< 0.1$ . Treatment and baseline value remained in the model regardless of their  $p$  values.

Variables were transformed (log or square root), if appropriate, to give the best estimate of a normal distribution, which was assessed by using the Shapiro-Wilk test. Non-inferiority for efficacy variables was concluded when the lower limit of the two-sided 95% confidence interval (CI) for the ratio of efficacy of IVP/Control was higher than  $1 - \delta$ , with  $\delta = 0.20$ . The selected  $\delta$  was based on the assumption that a difference of 20% or less in clinical scores between groups would not be relevant clinically. As most variables deviated significantly from a normal distribution in the RMANCOVA analyses, groups were compared at each assessed time by using the non-parametric Mann-Whitney or Kruskal-Wallis tests. Change from baseline was assessed by using the Wilcoxon signed-rank test. The occurrence of AEs was compared between groups by using the Fisher exact probability test.

For efficacy and safety assessments, the IVP group was compared separately to each of the Control I and Control II groups. In addition, the two control groups were compared and, if there was no significant difference, results from the two groups were combined (denoted Control I + II) and compared to those of the IVP group.

All statistical tests were two-sided on a 5% level of significance ( $\alpha = 0.05$ ) with no correction for multiple analyses, in order not to inflate the type II error.

A minimum inclusion of 60 dogs was planned, as was required by the Japanese regulatory authorities.

## Results

### Animals

The biological phase of the study ran from November 2013 to September 2014. A total of 67 dogs were enrolled in the study across 16 sites in Japan and all were included in the demographic and safety analyses, representing an "all-randomized animals" dataset. Age, body weight, gender, and breed were similar in the three groups (Table 3). A total of 15 breeds were included, the most common being Shih Tzu ( $n = 10$ ), Cavalier King Charles Spaniel ( $n = 9$ ), Chihuahua ( $n = 8$ ), Maltese ( $n = 8$ ), Miniature Dachshund ( $n = 5$ ), and mixed breed ( $n = 10$ ).

Two cases in the Control II group were removed from the efficacy dataset due to major protocol deviations (dosing of benazepril or pimobendan was  $\leq 80\%$  of scheduled). A total of 65 cases were therefore included in the efficacy dataset; all were fully compliant with the inclusion and exclusion criteria. Cases with missed doses (but with  $> 80\%$  scheduled dosing) or minor deviations from examination and observation schedules occurred in all three groups and were included in the efficacy analyses.

For the efficacy dataset, there were no differences between groups for age, body weight, breed, diet, or gender ( $p > 0.40$ ,

data not shown). At baseline, 56 dogs were in NYHA class III and 9 in class IV heart failure (Table 4).

#### Concomitant treatments and test items

Prior to D0, 53 dogs (79.1%) had previously received an ACEI, 33 dogs (49.3%) pimobendan, 19 dogs (28.4%) a diuretic, and 4 dogs (6.0%) amlodipine (Table 3). Six dogs had previously received ACEI, pimobendan, and diuretic, 12 ACEI and diuretic, 25 ACEI and pimobendan, 10 ACEI alone, 1 pimobendan alone, and 1 diuretic alone. With regard to ACEIs, dogs had received benazepril ( $n = 19$ ), enalapril ( $n = 14$ ),

**Table 3.** Baseline data and frequency of drug use

	IVP (n = 34)	Control I (n = 14)	Control II (n = 19)	p value
Age (yr)	12.1 $\pm$ 2.2 (6.0–15.1)	12.2 $\pm$ 2.7 (7.6–16.1)	11.9 $\pm$ 2.2 (8.0–15.8)	0.85
Body weight (kg)	7.0 $\pm$ 4.8 (2.7–25.6)	6.2 $\pm$ 3.0 (2.7–13.0)	5.8 $\pm$ 2.9 (2.5–13.5)	0.84
Gender				0.58
Male (unneutered)	8 (23.5)	7 (50.0)	5 (26.3)	
Male (neutered)	15 (44.1)	3 (21.4)	6 (31.6)	
Female (unneutered)	3 (8.8)	1 (7.1)	3 (15.8)	
Female (neutered)	8 (23.5)	3 (21.4)	5 (26.3)	
Previous treatment with				
ACEI	28 (82.4)	11 (78.6)	14 (73.7)	0.76
Pimobendan	16 (47.1)	7 (50.0)	10 (52.6)	0.93
Diuretic	5 (14.7)	6 (42.9)	8 (42.1)	0.044*
Furosemide	3 (8.8)	3 (21.4)	7 (36.8)	0.048*
Torasemide	1 (2.9)	2 (14.3)	2 (10.5)	0.34
Spironolactone	2 (5.9)	1 (7.1)	0 (0)	0.53
Amlodipine	2 (5.9)	1 (7.1)	1 (5.3)	0.97
Treatment in study with				
Diuretic	10 (29.4)	5 (35.7)	7 (36.8)	0.83
Furosemide	7 (20.6)	3 (21.4)	7 (36.8)	0.40
Torasemide	3 (8.8)	2 (14.3)	1 (5.3)	0.67
Spironolactone	2 (5.9)	1 (7.1)	0 (0)	0.53
Amlodipine	2 (5.9)	1 (7.1)	1 (5.3)	0.97

Data are presented as mean  $\pm$  SD (range) or number (%). IVP, investigational veterinary product; ACEI, angiotensin-converting enzyme inhibitor. \* $p < 0.05$ .

**Table 4.** Distribution of cases according to NYHA classification

		IVP			Control I			Control II		
		D0	D28	D56	D0	D28	D56	D0	D28	D56
NYHA class	I	0	1	0	0	0	0	0	0	0
	II	0	16	18	0	5	5	0	8	8
	III	31	17	14	12	9	8	13	9	8
	IV	3	0	2	2	0	1	4	0	1
Improvement rate (%)		55.9 (19/34)	58.8 (20/34)		42.9 (6/14)	35.7 (5/14)		52.9 (9/17)	52.9 (9/17)	

NYHA, New York Heart Association; IVP, investigational veterinary product; D, day.

temocapril (n = 13), alacepril (n = 6), or ramipril (n = 1) (Table 3). Mean (range) daily dosages in mg/kg/day administered prior to the study were: 0.59 (0.32–1.11) for benazepril, 0.75 (0.2–1.59) for enalapril, 0.16 (0.1–0.3) for temocapril, 3.4 (1.2–5.8) for alacepril, 0.1 (0.1–0.1) for ramipril, 0.53 (0.098–1.24) for pimobendan, and 0.38 (0.36–0.42) for amlodipine. Prior to the study, furosemide was used significantly more frequently in the

Control I and II groups than in the IVP group.

The mean (range) dosages of benazepril and pimobendan administered during the study are shown in Table 2. In the study, diuretics were used in 22 dogs (32.8%) and at similar dosages to those prior to enrollment in the study. The mean (range) daily dosages in mg/kg/day prior to and during the study were, respectively, 2.7 (0.44–4.8) and 3.0 (1.4–6.0) for furosemide

**Table 5.** *P* values for change from baseline analysis of efficacy endpoints

Variable	Day	<i>p</i> value			
		IVP	Control I	Control II	Control I + II
Primary endpoint					
Global clinical score	7	< 0.0001 D*	0.0156 D*	0.0020 D*	< 0.0001 D*
	14	< 0.0001 D*	0.0078 D*	0.0063 D*	< 0.0001 D*
	28	< 0.0001 D*	0.0977	0.0024 D*	0.0002 D*
	56	0.0046 D*	0.0938	0.0156 D*	0.0020 D*
Secondary endpoints					
Exercise tolerance	7	0.0313 D*	0.0625	0.0156 D*	0.0005 D*
	14	0.0088 D*	0.0313 D*	0.0156 D*	0.0002 D*
	28	0.0082 D*	0.0313 D*	0.0078 D*	0.0001 D*
	56	0.0098 D*	0.0313 D*	0.0156 D*	0.0002 D*
Demeanor	7	0.2500	0.2500	0.0625	0.0078 D*
	14	0.2656	0.2500	0.0781	0.0156 D*
	28	0.1309	1.0000	0.0313 D*	0.0371 D*
	56	0.0859	0.2500	0.0938	0.0234 D*
Appetite	7	0.1250	1.0000	0.0625	0.0313 D*
	14	0.4063	1.0000	0.0625	0.0313 D*
	28	0.5625	1.0000	0.0625	0.1250
	56	0.4844	1.0000	0.1250	0.1250
Respiratory effort	7	0.0107 D*	0.1250	0.1250	0.0137 D*
	14	0.0017 D*	0.1250	0.0469 D*	0.0044 D*
	28	0.0009 D*	0.1250	0.0234 D*	0.0020 D*
	56	0.0405 D*	0.2500	0.0859	0.0186 D*
Coughing	7	0.0005 D*	0.0313 D*	0.0156 D*	0.0002 D*
	14	0.0005 D*	0.0938	0.0156 D*	0.0011 D*
	28	0.0097 D*	0.2578	0.0039 D*	0.0029 D*
	56	0.0511	0.3438	0.0469 D*	0.0190 D*
Nocturnal dyspnea	7	0.0039 D*	0.2500	0.0313 D*	0.0039 D*
	14	0.0039 D*	0.1250	0.0625	0.0039 D*
	28	0.0010 D*	0.3750	0.0313 D*	0.0107 D*
	56	0.0625	0.6250	0.1875	0.0918
NYHA classification	28	< 0.0001 D*	0.0313 D*	0.0039 D*	< 0.0001 D*
	56	< 0.0001 D*	0.0625	0.0039 D*	0.0001 D*
FETCH	7	< 0.0001 D*	0.1714	0.0029 D*	0.0008 D*
	14	< 0.0001 D*	0.0454 D*	0.0043 D*	0.0002 D*
	28	< 0.0001 D*	0.0310 D*	0.0161 D*	0.0005 D*
	56	0.0020 D*	0.0332 D*	0.0015 D*	< 0.0001 D*
Pulmonary edema	28	< 0.0001 D*	0.0352 D*	0.0005 D*	< 0.0001 D*
	56	< 0.0001 D*	0.0039 D*	0.0137 D*	< 0.0001 D*

IVP, investigational veterinary product; D, decrease; NYHA, New York Heart Association; FETCH, functional evaluation of cardiac health. \**p* < 0.05.

and 0.19 (0.027–0.33) and 0.31 (0.1–0.55) for torasemide. Spironolactone was administered to 3 dogs (4.5%) at the same dosages (0.6, 1.33, and 1.95 mg/kg BID) before and during the study. Amlodipine was administered to 4 dogs (6.0%) at the same dosages (0.625 mg/kg BID  $n = 3$ , 1.25 mg/kg BID  $n = 1$ ) before and during the study.

#### Withdrawal of cases from the study

Four cases were withdrawn from the study before the scheduled D56 visit, all related to death or worsening of CHF. All 4 dogs had NYHA Class IV CHF at baseline. The cases were withdrawn on D52 (death from acute pulmonary edema) and D40 (worsening of CHF) in the IVP group ( $n = 2$ , 5.9%), on D52 (death from worsening of CHF) in Control I ( $n = 1$ , 7.1%), and on D51 (death from bronchopneumonia secondary to pulmonary edema) in Control II ( $n = 1$ , 5.3%). The withdrawal frequency was not different between groups ( $p = 0.98$ ). Since all 4 cases that were prematurely withdrawn related to a lack of efficacy of the test articles, values for GCS, body weight, BCS, pulmonary edema score, and NYHA class at the time of withdrawal were used in the analyses at later times based on the

last observation carried forward method [13].

#### Primary endpoint: global clinical score

There was no significant difference between the Control I and Control II groups for GCS ( $p = 0.46$ , RMANCOVA, data not shown) or any of the secondary variables; therefore, the two groups were combined to form the Control I + II group. There were significant decreases from baseline (D0) in GCS on all days (D7, D14, D28, and D56) in the IVP, Control II, and Control I + II groups (Table 5).

The RMANCOVA results for GCS revealed a significant effect of baseline ( $p < 0.0001$ ) correlating with the change from baseline, but no significant effect of treatment, previous and/or concomitant use of ACEI, pimobendan, diuretic, spironolactone, or amlodipine, time, or time  $\times$  treatment interaction (Table 6). Although use of diuretics prior to the trial was significantly different between groups (Table 3), this had no significant effect on any efficacy variable (Table 6). The RMANCOVA results also demonstrated no significant differences in GCS between the IVP group and the Control I ( $p = 0.91$ ), Control II ( $p = 0.17$ ), and combined Control I + II ( $p =$

**Table 6.** *P* values from the RMANCOVA model for efficacy endpoints

Variable	Transformation	Model effect <i>p</i> value										<i>p</i> value for normality	
		Treatment	Previous			Conco			Week	Trt $\times$ week	Baseline		
			ACEI	Pim	Diur	Diur	Spir	Aml					
Primary endpoint													
Global clinical score	Log	0.3762	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.3393	0.6975	< 0.0001*	0.0879
Secondary endpoints													
Exercise tolerance	None	0.1327	> 0.1	0.0328 I*	> 0.1	0.0550	> 0.1	0.0265 I*	0.1969	0.7004	< 0.0001*	0.0013*	
Demeanor	Square root	0.2084	> 0.1	> 0.1	> 0.1	0.0931	> 0.1	> 0.1	0.9682	0.6564	< 0.0001*	0.0049*	
Appetite	None	0.1729	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.3980	0.5485	0.0006*	< 0.0001*	
Respiratory effort	Log	0.5549	0.0924	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.0501	0.8679	< 0.0001*	0.0001*	
Coughing	None	0.8676	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.2837	0.8313	< 0.0001*	0.0003*	
Nocturnal dyspnea	None	0.9932	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.1746	0.8073	< 0.0001*	< 0.0001*	
NYHA classification	None	0.5566	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.4274	0.9975	0.5086	< 0.0001*	
FETCH	None	0.5458	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	0.0746	0.4057	0.6400	< 0.0001*	0.0284*	
Pulmonary edema	None	0.3046	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	NA	NA	0.1615	< 0.0001*	

RMANCOVA, repeated measures analysis of covariance; Conco, concomitant; ACEI, angiotensin-converting enzyme inhibitor; Pim, pimobendan; Diur, diuretic (not including spironolactone); Prev, previous; Spir, spironolactone; Aml, amlodipine; Trt, treatment; NYHA, New York Heart Association; FETCH, functional evaluation of cardiac health; I, increase; NA, not applicable. \* $p < 0.05$ .

0.33) groups. There were also no significant differences at any assessment time detected in the non-parametric Mann-Whitney analyses (data not shown). Non-parametric analyses are relevant since distribution of the GCS data approached significance from normality in the RMANCOVA analysis ( $p = 0.088$ ; Table 6).

Mean  $\pm$  SD GCS scores at D0 and D56 were  $12.1 \pm 3.1$  and  $10.6 \pm 3.6$  in the IVP group;  $11.5 \pm 4.0$  and  $10.1 \pm 4.1$  in Control I; and  $13.7 \pm 5.0$  and  $10.1 \pm 4.0$  in Control II, respectively.

Results of the non-inferiority analysis are shown in Table 7. Since the lower limit of the 95% CI for the ratio of efficacy of IVP/Control was higher than 0.80, non-inferiority of the IVP was demonstrated when compared to the Control I, Control II, and combined Control I + II groups.

### Secondary efficacy endpoints

There were significant decreases from baseline (D0) in NYHA class, FETCH and pulmonary edema scores, as well as in some of the other variables in the IVP and control groups (Table 5).

In the RMANCOVA analyses, no significant overall treatment effect for any secondary variable was detected (Table 6). In the non-parametric analyses using the Mann-Whitney  $U$  test, no statistically significant differences were observed for any of individual clinical scores, NYHA class, and the FETCH and pulmonary edema scores at any of the assessment times between the groups (IVP vs. Control I, II, and I + II, and Control I vs. II,  $p > 0.05$ , data not shown). The results of the non-parametric analyses are relevant since the distributions of

all 8 secondary variables deviated significantly from normality in the RMANCOVA analyses (Table 6).

The NYHA classification distribution is shown in Table 4; the improvement rate from baseline ranged from 35.7% to 58.8%. There was no significant treatment effect ( $p = 0.56$ ; Table 6), and non-inferiority was shown for the IVP group vs. Control I, II, and I + II groups (Table 7).

With regard to pulmonary edema scores, there was a significant change (improvement) from D0 to both D28 and D56 in all groups: IVP from  $2.24 \pm 0.50$  to  $1.56 \pm 0.61$  and  $1.65 \pm 0.73$ ; Control I from  $2.29 \pm 0.61$  to  $1.7 \pm 0.61$  and  $1.57 \pm 0.65$ ; and Control II from  $2.41 \pm 0.71$  to  $1.41 \pm 0.51$  and  $1.65 \pm 0.79$ , respectively. There was no significant overall treatment effect ( $p = 0.30$ ; Table 6), and non-inferiority was demonstrated for the IVP vs. the Control I and combined Control I + II groups (Table 7).

### Safety

All 67 dogs in the all-randomized animal dataset were included in the safety analyses.

**Adverse events:** There were no significant differences between groups in the frequency of all AEs: 17 cases (50.0%) in the IVP group, 11 cases (78.6%) in Control I, 12 cases (63.2%) in Control II, and 23 cases (69.7%) in Control I + II (Table 8).

The only AE with a significant difference between groups was emesis, which was significantly less frequent in the IVP group than in the Control I ( $p = 0.0034$ ) and Control I + II ( $p = 0.0042$ ) groups; significance was approached ( $p = 0.056$ ) for the difference in emesis between the IVP and Control II groups.

**Table 7.** Non-inferiority analysis using RMANCOVA for efficacy endpoints

Variable	Relative efficacy					
	IVP vs. Control I		IVP vs. Control II		IVP vs. Control I + II	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Primary endpoint						
Global clinical score	0.992	0.867*–1.135	0.917	0.808*–1.040	0.950	0.856*–1.055
Secondary endpoints						
Exercise tolerance	0.993	0.852*–1.192	0.844	0.729–1.004	0.913	0.806*–1.054
Demeanor	1.005	0.830*–1.243	0.846	0.706–1.033	0.917	0.787–1.082
Appetite	1.010	0.892*–1.164	0.887	0.787–1.017	0.945	0.855*–1.056
Respiratory effort	0.953	0.803*–1.131	0.919	0.784–1.077	0.934	0.818*–1.066
Coughing	0.949	0.797–1.173	0.986	0.837*–1.198	0.969	0.845*–1.136
Nocturnal dyspnea	1.001	0.851*–1.214	1.009	0.867*–1.208	1.005	0.885*–1.163
NYHA classification	1.069	0.949*–1.223	1.016	0.908*–1.155	1.040	0.946*–1.155
FETCH	1.033	0.806*–1.439	0.872	0.688–1.191	0.947	0.775*–1.216
Pulmonary edema	1.014	0.823*–1.250	0.911	0.747–1.110	0.957	0.812*–1.128

Results are the ratio of the reciprocal of variables for IVP/Control; therefore, values  $> 1$  indicate superior efficacy of the IVP and values  $< 1$  superior efficacy of the control treatment. RMANCOVA, repeated measures analysis of covariance; IVP, investigational veterinary product; CI, confidence interval; NYHA, New York Heart Association; FETCH, functional evaluation of cardiac health. \*Non-inferiority shown (lower limit of 95% CI  $> 0.80$ ).

**Table 8.** Frequency of reported adverse events

System/preferred term	Frequency			<i>p</i> value for comparison of IVP vs.		
	IVP (n = 34)	Control I (n = 14)	Control II (n = 19)	Control I	Control II	Control I + II
Cardiovascular system	2	0	1	0.58	1.0	1.0
Digestive tract	11	9	8	0.057	0.56	0.14
Diarrhea	10	4	2	1.0	0.17	0.39
Emesis	3	7	6	0.0034*	0.056	0.0042*
Neurological	4	1	0	1.0	0.28	0.36
Ataxia	2	0	0	0.58	0.53	0.49
Muscle tremor	2	0	0	0.58	0.53	0.49
Respiratory tract	5	0	3	0.30	1.0	0.71
Cough	3	0	0	0.55	0.30	0.24
Pulmonary edema	2	0	1	0.58	1.0	1.0
Rhinitis	0	0	2	1.0	0.12	0.24
Skin and appendages	1	1	0	1.0	1.0	1.0
Systemic	4	4	5	0.21	0.26	0.13
Anorexia	3	3	3	0.34	0.65	0.31
Death	1	1	1	1.0	1.0	0.61
Any sign	17	11	12	0.11	0.40	0.14

Only adverse events reported in at least 2 dogs are shown. IVP, investigational veterinary product. \**p* < 0.05.

The number (%) of dogs with emesis was 3 (8.8%) in the IVP group, 7 (50.0%) in Control I, 6 (31.6%) in Control II, and 13 (39.4%) in Control I + II.

**Clinical chemistry:** With the exception of an effect on plasma glucose, there was no significant overall treatment effect or differences between the IVP and Control I or Control II groups for any clinical chemistry or hematology variable (data not shown). The *p* values for the overall treatment effect in the RMANCOVA model did not approach significance for the most clinically relevant variables: alanine aminotransferase (*p* = 0.80), creatinine (*p* = 0.68) or urea (*p* = 0.64). For potassium, there were numerically higher concentrations (mEq/L) in the Control I (5.1 ± 0.5) and Control II (5.0 ± 0.5) groups vs. the IVP group (4.9 ± 0.6), but the overall treatment effect was not significant (*p* = 0.12). For glucose, values (mg/dL) were significantly (*p* = 0.016) higher in the IVP group (92.9 ± 17.6) than in the Control II group (78.9 ± 15.4). Regardless, mean values for glucose and potassium remained within the reference range in all groups.

For plasma creatinine, the number of cases with increases from baseline by > 0.3 mg/dL, > 20%, or > 30% were calculated. There were no significant treatment effects or differences between groups. At day 28, the number of cases, respectively, in the IVP, Control I, and Control II groups with increases from baseline by > 0.3 mg/dL was 3, 0, and 4 (*p* = 0.14); by > 20% was 8, 2, and 7 (*p* = 0.32); and by > 30% was 5, 0, and 5 (*p* = 0.11).

**Other:** There was no significant overall treatment effect or

differences between groups in the results of the RMANCOVA or non-parametric analyses for BCS and body weight (data not shown).

## Discussion

The aim of this study was to compare the field efficacy and safety of the new combination tablet of benazepril and pimobendan (Fortekor Plus) to the reference products (Fortekor and Vetmedin) in order to support the registration of Fortekor Plus in Japan. The number of dogs recruited (67) was determined by a regulatory requirement of a minimum of 60 cases. The study achieved its objective by demonstrating non-inferior efficacy and good safety of Fortekor Plus compared to the reference products in dogs with CHF caused by MMVD.

It has been shown in bioequivalence studies that plasma concentrations of benazepril, pimobendan, and their metabolites do not exhibit biologically relevant differences after administration of Fortekor Plus tablets from those of Fortekor and Vetmedin in healthy beagle dogs (Elanco Animal Health; unpublished data). Regardless, field testing of Fortekor Plus was important for several reasons. First, bioequivalence studies may not detect all clinically relevant differences in efficacy or safety. Second, the weight bands in the dosing charts for Fortekor and Vetmedin differ, and the maximum daily dosage for pimobendan in Fortekor Plus (0.5 mg/kg) is slightly lower than for Vetmedin in Japan (0.7 mg/kg). In addition, for

benazepril in Fortekor, the minimum starting dosage of 0.25 mg/kg SID can be doubled to 0.5 mg/kg SID. Since no doubling of the dosage of benazepril can be accommodated by using Fortekor Plus, as it is not permitted for pimobendan in Vetmedin, the upper end of the dosage range for benazepril, *i.e.*, 0.5 to 1.0 mg/kg/day, is recommended for Fortekor Plus. In this study, the dose regimens intended for Fortekor Plus were compared to those presently registered for Fortekor and Vetmedin in Japan. As a consequence, in the IVP group compared to the control groups, the daily mean dosage of benazepril was higher (respectively, 0.72 *vs.* 0.52 mg/kg) and the dosage of pimobendan slightly lower (0.36 *vs.* 0.48 mg/kg). It should be noted that the dosage ranges for pimobendan tested in this study (IVP, 0.26–0.5 mg/kg/day; control groups, 0.36–0.6 mg/kg/day) are lower than those that were tested in the QUEST and EPIC studies (0.4–0.6 mg/kg/day) [5,11]. Third, benazepril is registered with SID dosing in Fortekor, but needs to be administered BID in Fortekor Plus in order to be compatible with pimobendan. Effects of BID and SID dosing of benazepril have been compared in healthy dogs by examining plasma or serum ACE activity, but those results do not predict all actions of ACEIs in clinical cases [19]. Therefore, in this study, we compared SID and BID dosing of benazepril, in combination with pimobendan, via the use of two control groups. There were no significant differences in efficacy variables between groups and non-inferiority was demonstrated between the IVP and the control groups (both analyzed separately and combined) for the primary endpoint, indicating no clinically relevant differences in efficacy between the test articles and the dosage regimens.

Dogs in the IVP and both control groups showed significant improvement from baseline for the GCS and some secondary endpoints including NYHA class, and FETCH and pulmonary edema scores. This can be explained by the fact that all dogs were treated with an ACEI (benazepril) and pimobendan after D0, whereas prior to D0 only 79.1% of dogs received an ACEI and 49.3% received pimobendan, reflective of first opinion practice in Japan. Frequency of use and dosages of amlodipine, furosemide, spironolactone, and torasemide were similar prior to and after D0. The improvement *vs.* baseline in all groups provides some evidence that benazepril and pimobendan in combination are effective in improving clinical signs in dogs with CHF caused by MMVD. To our knowledge, the combination of benazepril and pimobendan in dogs with CHF has not been tested previously in controlled prospective studies, although their combined use in a retrospective study has been reported [9].

An important limitation of the study is that the primary and secondary efficacy endpoints relied on subjective assessments of the dog's clinical condition, made by an investigator after examining the dog and questioning the owner. We employed the same scoring schemes as were used previously in the QUEST

and EPIC studies [5,11]. Since there was no negative control group to provide internal validity, there is nevertheless a risk that the efficacy of all test articles was overestimated via the well-established “caregiver placebo effect” [8]. In addition, the owners were not blinded to the test articles and there is a risk that they could have influenced the assessments made by the veterinarians. The pulmonary edema results are useful, therefore, since their assessment (of radiographs using a standard scheme) was more objective than the subjective scoring assessments. The pulmonary edema results support the conclusions of the other efficacy endpoints, with clinically relevant and significant improvement from baseline in all groups and non-inferior efficacy of the IVP compared to the Control I and Control I + II groups.

The safety of Fortekor Plus at its recommended dosing regimen was at least as good as that associated with the administration of Fortekor and Vetmedin. The frequency of all AEs was lower in the IVP group (50.0%) than in the control groups (78.6% and 63.2%), but the differences were not significant ( $p = 0.14$ ). Most of the reported AEs were likely associated with the primary disease (CHF) or were incidental and not specifically related to the administration of IVP or control drugs. The frequency of emesis was lower in the IVP group (8.8%) than in the control groups (50.0% and 31.6%), and the magnitude of difference was clinically relevant and highly significant ( $p < 0.01$ ). Reduced emesis could be the result of fewer tablets dosed and/or different excipients in Fortekor Plus compared to those in Fortekor and Vetmedin.

With the exception of plasma glucose, which could have indicated a type I error considering the multiple analyses, there were no significant differences between groups for any clinical chemistry or hematology variable. For plasma creatinine, the  $p$  values did not approach significance for overall treatment effect ( $p = 0.68$ ) or comparisons between groups ( $p \geq 0.52$ ). These results suggest no marked differences between groups in the risk of acute kidney injury, which might have been of concern considering the higher dosage of benazepril in the IVP group. There is also the theoretical risk that a higher dosage of benazepril could lead to a higher frequency of hyperkalemia. There were, however, no significant differences between groups in plasma potassium concentrations, no cases in any group with marked increases in concentrations, and the mean values descended in the order Control I > Control II > IVP. Therefore, no special risks related to plasma creatinine or potassium were associated with Fortekor Plus treatment.

As noted previously, the daily dosage of benazepril is higher in Fortekor Plus compared to Fortekor, and it is administered BID. The results from the present study suggest no disadvantages for either efficacy or safety with the higher daily benazepril dosage of 0.72 mg/kg, administered in the IVP group, compared to the 0.50 or 0.54 mg/kg dosages used in the Control I or II groups. Toutain *et al.* [19] predicted, from

modeling, that administration of benazepril at half of the dosage BID should inhibit plasma ACE activity over 24 h at least as well as the full dosage SID, and this suggestion was confirmed recently by King *et al.* (unpublished paper) examining serum ACE. In the BENCH study, doubling of the dosage of benazepril from 0.25 to 0.5 mg/kg SID to 0.5 to 1.0 mg/kg SID did not increase the frequency of reported AEs [3,4]. However, no conclusions on relative efficacy of different dosages of benazepril can be made from the BENCH study due to the study design.

In addition to those mentioned previously, the principal limitations of the present study are discussed below.

First, a study population size of 67 dogs is relatively low for a field study. The sample size was determined by the regulatory requirement for a minimum of 60 cases, and proved to be sufficient to test the primary objective of the study, *i.e.*, demonstration of non-inferior efficacy for the primary endpoint with the defined  $\delta$  of 20%. The limited number of dogs means that the study had low power to detect subtle differences in efficacy between the groups or to detect uncommon AEs; for example, the study had only 49% power to detect AEs with a true incidence of 1%.

Second, statistical analysis of the efficacy data was a challenge since ordinal scores were used and assessed repeatedly. To our knowledge, good non-parametric statistical methods for testing non-inferiority are not available. Therefore, the data were analyzed primarily by applying the parametric RMANCOVA method. Despite transforming data where appropriate, the distribution of all secondary endpoints deviated significantly from normality in the RMANCOVA analyses, and the distribution of the primary endpoint approached significance ( $p = 0.088$ ). However, ANOVA models are relatively robust even in the absence of normality [12]; therefore, the results of the RMANCOVA analyses are judged to be reliable. Furthermore, the conclusion of lack of significant differences between groups for efficacy variables was confirmed by non-parametric analyses.

Third, we studied dogs at Japanese clinics with CHF in NYHA classes III and IV caused by MMVD. Diuretics were used in fewer cases (~30%) than is standard in many other countries, even though all dogs had evidence of pulmonary edema at baseline. The results, therefore, cannot be simply generalized to other settings.

Fourth, it was noted that furosemide was used prior to the study significantly more frequently in Control I and II groups than in the IVP group. Although this might have resulted from differences in disease severity at baseline, there was no detected effect on study conclusions as there was no significant association between previous use of diuretics and outcomes for any endpoint (Table 6).

Fifth, limitations of non-inferiority studies have been well described, notably that the absence of significant differences

between groups can be due to similar efficacy or similar lack of efficacy if there is no negative control to provide internal validity [17]. However, the significant improvement from baseline in multiple variables, including the GCS and pulmonary edema scores, supports the conclusion that the IVP and both control groups had similar beneficial efficacy.

Finally, our study did not address in any way the possible advantages of using an ACEI with pimobendan over those of either drug alone. Further studies into the combination of benazepril and pimobendan are recommended.

## Acknowledgments

We thank Drs. Toshihiro Aoki, Ryo Baba, Shuichi Chimura, Maya Furukoshi, Jinpei Honjo, Mitsuhiro Isaka, Fumie Kawai, Kazuhito Kodama, Toru Kurita, Shigeki Maetani, Takahiro Mizukoshi, Hiroshige Mizuno, Toshiaki Mogi, Takuro Mori, Takashi Morishima, Shigeru Nakamura, Tatsuya Oyama, Kenji Rinpou, Yuzuru Sakai, Tamotsu Sawada, Asako Shinoda, Masami Uechi, Shigeki Yamano, Hidemi Yasuda, and Yuji Yoshinaga for managing the clinical cases, and Kenji Oda, Yumi Nishida, and staff of the Research Institute for Animal Science in Biochemistry and Toxicology, Kanagawa, Japan for monitoring the study.

## Conflict of Interest

This study was funded by, and JN King, J Sonobe, H Otaki, N Sakakibara, W Seewald, and S Forster are employed by, Elanco Animal Health which markets benazepril (Fortekor) and the combination of benazepril plus pimobendan (Fortekor Plus).

## References

1. **Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R.** Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *J Vet Intern Med* 2009, **23**, 1142-1150.
2. **Bangalore S, Kamalakkannan G, Parkar S, Messerli FH.** Fixed-dose combinations improve medication compliance: a meta-analysis. *Am J Med* 2007, **120**, 713-719.
3. **BENCH (BENazepril in Canine Heart disease) Study Group.** The effect of benazepril on survival times and clinical signs of dogs with congestive heart failure: Results of a multicenter, prospective, randomized, double-blinded, placebo-controlled, long-term clinical trial. *J Vet Cardiol* 1999, **1**, 7-18.
4. **BENCH (BENazepril in Canine Heart disease) Study Group, Pouchelon JL, King J, Martignoni L, Chetboul V, Lugardon B, Rousselot JF, Corlouer JP, Bussadori C, Piette MH, Brownlie S, Martel P, Garcin JP, Hagen A, Amberger C, Martin M, Labadie F, Collet M, Drouard C, Lombard C, Hervé D, Strehlau G.** Long-term tolerability of benazepril in

- dogs with congestive heart failure. *J Vet Cardiol* 2004, **6**, 7-13.
5. **Boswood A, Häggström J, Gordon SG, Wess G, Stepien RL, Oyama MA, Keene BW, Bonagura J, MacDonald KA, Patteson M, Smith S, Fox PR, Sanderson K, Woolley R, Szatmári V, Menaut P, Church WM, O'Sullivan ML, Jaudon JP, Kresken JG, Rush J, Barrett KA, Rosenthal SL, Saunders AB, Ljungvall I, Deinert M, Bomassi E, Estrada AH, Fernandez Del Palacio MJ, Moise NS, Abbott JA, Fujii Y, Spier A, Luethy MW, Santilli RA, Uechi M, Tidholm A, Watson P.** Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: the EPIC study—a randomized clinical trial. *J Vet Intern Med* 2016, **30**, 1765-1779.
  6. **Boyle KL, Leech E.** A review of the pharmacology and clinical uses of pimobendan. *J Vet Emerg Crit Care (San Antonio)* 2012, **22**, 398-408.
  7. **Claxton AJ, Cramer J, Pierce C.** A systematic review of the associations between dose regimens and medication compliance. *Clin Ther* 2001, **23**, 1296-1310.
  8. **Conzemius MG, Evans RB.** Caregiver placebo effect for dogs with lameness from osteoarthritis. *J Am Vet Med Assoc* 2012, **241**, 1314-1319.
  9. **de Madron E, King JN, Strehlau G, White RV.** Survival and echocardiographic data in dogs with congestive heart failure caused by mitral valve disease and treated by multiple drugs: a retrospective study of 21 cases. *Can Vet J* 2011, **52**, 1219-1225.
  10. **Freeman LM, Rush JE, Farabaugh AE, Must A.** Development and evaluation of a questionnaire for assessing health-related quality of life in dogs with cardiac disease. *J Am Vet Med Assoc* 2005, **226**, 1864-1868.
  11. **Häggström J, Boswood A, O'Grady M, Jöns O, Smith S, Swift S, Borgarelli M, Gavaghan B, Kresken JG, Patteson M, Ablad B, Bussadori CM, Glaus T, Kovacević A, Rapp M, Santilli RA, Tidholm A, Eriksson A, Belanger MC, Deinert M, Little CJ, Kvart C, French A, Rønn-Landbo M, Wess G, Eggertsdóttir AV, O'Sullivan ML, Schneider M, Lombard CW, Dukes-McEwan J, Willis R, Louvet A, DiFruscia R.** Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: the QUEST study. *J Vet Intern Med* 2008, **22**, 1124-1135.
  12. **Khan A, Rayner GD.** Robustness to non-normality of common tests for the many-sample location problem. *J App Math Dec Sci* 2003, **7**, 187-206.
  13. **Kim Y.** Missing data handling in chronic pain trials. *J Biopharm Stat* 2011, **21**, 311-325.
  14. **King JN, Mauron C, Kaiser G.** Pharmacokinetics of the active metabolite of benazepril, benazeprilat, and inhibition of plasma angiotensin-converting enzyme activity after single and repeated administrations to dogs. *Am J Vet Res* 1995, **56**, 1620-1628.
  15. **Lombard CW, Jöns O, Bussadori CM.** Clinical efficacy of pimobendan versus benazepril for the treatment of acquired atrioventricular valvular disease in dogs. *J Am Anim Hosp Assoc* 2006, **42**, 249-261.
  16. **Mochel JP, Peyrou M, Fink M, Strehlau G, Mohamed R, Giraudel JM, Ploeger B, Danhof M.** Capturing the dynamics of systemic Renin-Angiotensin-Aldosterone System (RAAS) peptides heightens the understanding of the effect of benazepril in dogs. *J Vet Pharmacol Ther* 2013, **36**, 174-180.
  17. **Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ; CONSORT Group.** Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. *J Am Med Assoc* 2006, **295**, 1152-1160.
  18. **Smith PJ, French AT, Van Israël N, Smith SG, Swift ST, Lee AJ, Corcoran BM, Dukes-McEwan J.** Efficacy and safety of pimobendan in canine heart failure caused by myxomatous mitral valve disease. *J Small Anim Pract* 2005, **46**, 121-130.
  19. **Toutain PL, Lefebvre HP, King JN.** Benazeprilat disposition and effect in dogs revisited with a pharmacokinetic/pharmacodynamic modeling approach. *J Pharmacol Exp Ther* 2000, **292**, 1087-1093.