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# Clinical efficacy of tadalafil compared to sildenafil in treatment of moderate to severe canine pulmonary hypertension: a pilot study☆

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**KEYWORDS**
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**Abstract** *Introduction:* Canine pulmonary hypertension (PH) is associated with high morbidity and mortality. Tadalafil, a phosphodiesterase-5 inhibitor used commonly in humans with PH, has not been evaluated in a clinical trial in dogs with naturally occurring PH. Our objectives were to compare the efficacy of tadalafil and sildenafil on PH assessed by peak tricuspid regurgitant flow velocity, estimated systolic pulmonary arterial pressure gradient, voluntary activity, quality of life, and safety profiles in dogs with moderate to severe PH.

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*Animals:* Twenty-three dogs with echocardiographic evidence of moderate to severe PH were enrolled.
*Methods:* A prospective short-term, randomized, double-blinded pilot study was carried out. Dogs with PH were randomly allocated to receive sildenafil or tadalafil for 2 weeks and assessed via echocardiography, activity monitors, and owner-reported outcomes.
*Results:* Collectively, phosphodiesterase-5 inhibition significantly decreased (improved) quality of life scores ($p = 0.003$) and visual analog score ($p = 0.024$) without significant between-treatment difference of these variables. Phosphodiesterase-5 inhibition did not significantly affect peak tricuspid regurgitant flow velocity ($p = 0.056$) or voluntary activity ($p = 0.27$). A total of 33% (7/21) of dogs experienced at least one adverse event during the study (tadalafil, n = 5; sildenafil, n = 2) with no significant difference between treatment type and incidence of adverse events ($p = 0.36$).
*Discussion:* In this pilot study, phosphodiesterase-5 inhibition led to apparent improvement in quality of life scores without documenting superiority of tadalafil over sildenafil.
*Conclusion:* Tadalafil at a dose of 2 mg/kg once daily appears to be a viable alternative to sildenafil in dogs with moderate to severe PH.


**Abbreviations**

| | |
|---|---|
| AEs | adverse events |
| AEB | afternoon and evening bottle |
| ANOVA | analysis of variance |
| AM | activity monitor |
| AT:ET | acceleration time indexed to the ejection time of pulmonary artery flow |
| CHF | congestive heart failure |
| FETCH | functional evaluation of cardiac health |
| MB | morning bottle |
| PAP | pulmonary arterial pressure |
| PDE5 | phosphodiesterase-5 |
| PDE5I | phosphodiesterase-5 inhibitor |
| PH | pulmonary hypertension |
| QOL | quality of life |
| RPAD | right pulmonary artery dispensability index |
| SABP | systolic arterial blood pressure |
| sPAP | systolic pulmonary arterial pressure |
| TRFV | tricuspid regurgitant flow velocity |
| VAS | visual analog score |

## Introduction

Pulmonary hypertension (PH) is characterized by persistently increased pulmonary arterial pressure (PAP), which can lead to pulmonary arterial remodeling, right ventricular failure, and death [1]. Canine PH has been defined as pulmonary arterial systolic pressure >30 mmHg estimated with echocardiography [2]. In dogs, PH can be primary (idiopathic) or secondary to left-sided heart disease [3—5], respiratory disease [6—10], left-to-right cardiovascular shunts [6—9], heartworm disease [6,7,11], and pulmonary thromboembolism [6—8]. Pulmonary vascular pathology and disease progression are shared sequela, regardless of disease etiology [12]. The nitric oxide pathway is impaired by upregulated phosphodiesterase-5 (PDE5) in pulmonary vessels which decreases cyclic guanosine monophosphate contributing to endothelial dysfunction and smooth muscle cell proliferation [12].

Therapy includes addressing identified underlying causes and administration of a PDE5 inhibitor (PDE5I) when indicated. Phosphodiesterase-5 inhibitors prevent cyclic guanosine monophosphate metabolism, prolonging protein kinase G activation. This decreases pulmonary artery smooth muscle cell calcium concentration, causing pulmonary artery vasodilation and decreased smooth muscle cell proliferation [13]. Currently, sildenafil is the only PDE5I routinely used in canine PH. While sildenafil improves clinical signs related to PH [8,14], its short half-life in dogs (3—4 h) [15,16] requires dosing three times daily, which can compromise treatment compliance and owner—dog relationship. Additionally, its long-term costs may preclude treatment.

Tadalafil is a newer oral PDE5I for treatment of pulmonary arterial hypertension in people [17]. Tadalafil and sildenafil have similar efficacy and safety profiles in people with pulmonary arterial hypertension [18—20]. Several pharmacokinetic

and pharmacodynamic differences between these drugs could make tadalafil a better alternative. For example, tadalafil in people has a long plasma half-life (17.5 h), facilitating once daily dosing [21]. While tadalafil and sildenafil inhibit P-glycoprotein in vitro, inhibition is less with tadalafil (66%) versus sildenafil (99%) [22] potentially resulting in fewer drug—drug interactions and drug toxicities. Tadalafil is markedly more selective for PDE5 than sildenafil and may have fewer adverse effects from a lower proclivity to inhibit unintended PDE isoenzymes [12].

While tadalafil attenuates PAP following a single dose in experimentally induced PH in dogs [23], clinical use for naturally occurring PH in dogs is limited to a single case report [24]. The study objective was to compare efficacy of tadalafil with sildenafil on peak tricuspid regurgitant flow velocity (TRFV), estimated systolic pulmonary arterial pressure (sPAP), voluntary activity, quality of life (QOL), and safety profiles in moderate to severe canine PH. We hypothesized that dogs with moderate to severe PH treated with tadalafil would exhibit greater decreases in peak TRFV and estimated sPAP and significantly greater increases in voluntary activity, QOL, and fewer adverse events (AEs) compared to sildenafil.

## Animals, materials and methods

### Study design and animals

Dogs of any age, breed, or sex were eligible for enrollment in this prospective, randomized, double-blinded pilot study between August 2017 and February 2018. The study protocol was approved by the University of Missouri Veterinary Health Center Animal Care and Use Committee (protocol #8916) with written owner consent. Dogs were examined on days 0 and 15, and questionnaires and visual analog scores (VASs) were assessed on days 0, 8, and 15. A power calculation *a priori* was performed utilizing data extrapolated from the only clinical trial directly comparing the effect (i.e. change in mean PAP) of tadalafil with sildenafil over time in humans [25]. The difference between means in that study (1.81 mmHg) and largest variance (8.6) with a $\beta = 0.20$ and $\alpha = 0.05$ indicated a sample size of 356 dogs would be needed to detect a significant difference in our study [25]. Understanding this sample size would not be easily achievable; we instead used a similar population size to a study investigating clinical efficacy of sildenafil in dogs with myxomatous mitral valve disease (effective population after cross-over, n = 16) [14]. We increased the number to 23 to account for disease etiology variation and potential dropout.

### Inclusion and exclusion criteria

Client-owned dogs with clinical signs of chronic cough, tachypnea, respiratory distress, cyanosis, or syncope or in which thoracic auscultation revealed abnormal sounds (i.e. crackles or wheezes) or a heart murmur, or dogs with positive heartworm tests were screened for enrollment at the University of Missouri Veterinary Health Center. To be enrolled, each dog had to have echocardiographic abnormalities suggestive of moderate to severe PH including TRFV$\geq$3.5 m/s, evidence of Eisenmenger physiology, or at least two of the following: type III pulmonary flow profiles (i.e. notched), the right pulmonary artery distensibility index (RPAD) $<$ 29.5%, or the ratio of Doppler-derived acceleration time indexed to ejection time of pulmonary artery flow (AT:ET) $<$ 0.30 [3,26]. Dogs were categorized into 5 groups based on clinical PH classification in people [27]: pulmonary arterial hypertension (group 1), PH due to left heart disease (group 2), PH due to chronic lung disease and/or hypoxia (group 3), chronic thromboembolic PH (group 4), and PH due to unclear or multifactorial mechanisms (group 5). Stratification of dogs into these groups was based on diagnostics performed by attending clinicians and subject to owner approval and finances. Not all dogs received the same diagnostic tests. Dogs were grouped within those aforementioned constraints. The diagnosis (and thus grouping) was performed at the highest level of available knowledge by a boarded cardiologist (S.B.L.) and internists (C.R.R., J.A.J.) Exclusion criteria were pulmonic stenosis, radiographic evidence suggestive of pulmonary edema and acute left-sided congestive heart failure (CHF), systemic hypotension ($<$90 mmHg via Doppler ultrasound blood pressure), conditions requiring surgical intervention, or when primary clinicians perceived great risk for imminent non-survival. Concurrent therapy for chronic CHF with diuretics, angiotensin-converting enzyme inhibitors, and pimobendan and other medications for comorbid conditions were permitted if initiated $>$1 week prior to enrollment and dosages remained unchanged during study. Previous treatment with sildenafil was permitted, provided there was a washout of 7 days and echocardiographic evidence of moderate to severe PH persisted after the

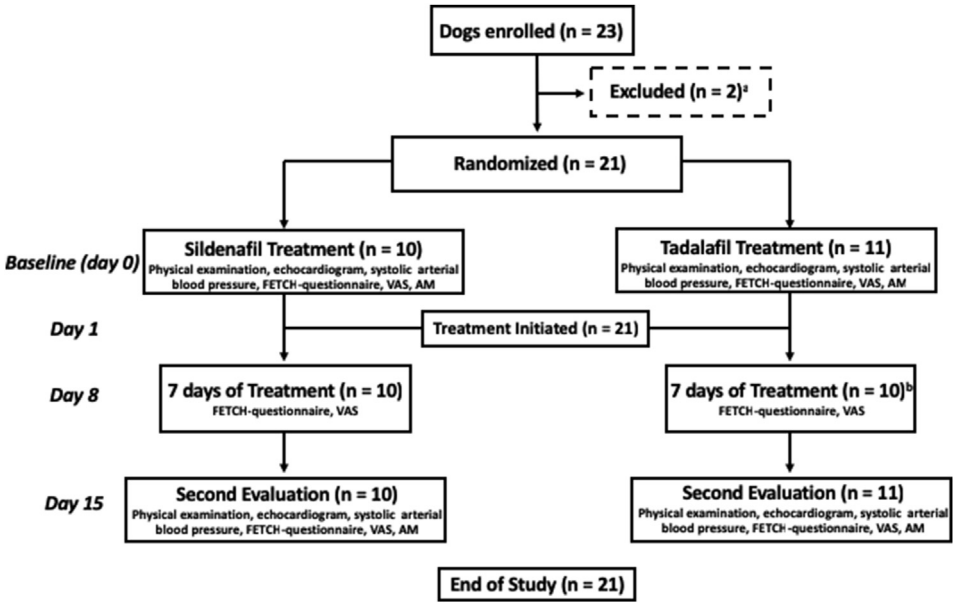


**Fig. 1** Flowchart documenting the numbers of dogs recruited to each treatment and their progress through the trial. [a]Two dogs were excluded for 1 each of the following: death from severe underlying respiratory disease at home shortly after the study and pacemaker lead migration with sick sinus syndrome. [b] One dog diagnosed with pneumocystis pneumonia in the tadalafil treatment group was hospitalized for an extended period of time and thus did not have FETCH questionnaire or VAS score assessment by the owner at week 1 (day 8) or at the final evaluation. FETCH, functional evaluation of cardiac health.

washout. During this brief washout, owners were instructed to monitor for lethargy, resting respiratory rate >40 breaths/minute, labored respiration, and syncope. If these clinical signs occurred, sildenafil was to be restarted and the primary clinician was contacted immediately. Dogs could be withdrawn from study at the owner's request if systolic arterial blood pressure (SABP) decreased below 90 mmHg or if the dog's condition deteriorated with concern for mortality.

## Randomization, drug preparation, administration, and masking

Dogs were randomized via a computer-generated schedule into one of the 2 treatments (Fig. 1). Randomization, drug compounding, and dispensation were performed by a pharmacist or a technician under the direct supervision of a pharmacist blinded to the dog characteristics other than body weight. Dogs receiving sildenafil had a compounded suspension allocated into 2 bottles labeled 'morning bottle' (MB) and 'afternoon and evening bottle' (AEB) at a dose of 0.5 mg/kg (1 mL/dose) for a total of 3 doses every 8 h for 14 days. Dogs receiving tadalafil had a compounded suspension allocated into a bottle labeled MB and placebo suspension allocated into a bottle labeled AEB. These dogs received tadalafil at a dosage of 2 mg/kg (1 mL/dose) from the MB in the morning and 2 administrations of placebo (1 mL/dose) from the AEB with 8 h between doses for 14 days. Treatment in both groups was started the morning after PH diagnosis (day 1).

Sildenafil, tadalafil, and placebo were matched with regard to suspension consistency, color, and volume. Owners, veterinarians, and all clinical personnel participating in evaluations were masked to treatment. Treatment code was known only to the pharmacist treatment dispenser. All owners were requested to return medication bottles at the treatment period end. Dosing compliance was evaluated by measuring the residual volume from MB and AEB when available for each dog at the study end and comparing it with what was dispensed.

## Outcome measures

### Hemodynamics

Echocardiography was performed using a conventional echocardiographic system and phased array transducers with nominal frequencies ranging from 2.0 to 8.5 MHz.[e] All studies were

[e] Aplio Artida, Toshiba Medical Systems Corporation, Otawara, Japan.

performed by a board-certified veterinary cardiologist or cardiology resident under supervision of a board-certified veterinary cardiologist utilizing standard views in unsedated dogs restrained in lateral recumbency [28]. Echocardiograms were interpreted by group consensus (S.B.L., L.R.K., and K.E.W.). Sonographers were blinded to the treatment group but not to time point (i.e. baseline versus final evaluation). Tricuspid and pulmonic valves were imaged in multiple planes to optimize alignment for Doppler interrogation of regurgitant jets and to evaluate pulmonary artery systolic flow velocity and profile. Estimated systolic and mean pressure gradients were calculated by applying the modified Bernoulli equation to maximal velocity of tricuspid and pulmonic regurgitant jets, respectively. Right atrial pressure was estimated at 5 mmHg with subjectively normal right atrial size, 10 mmHg with a subjectively enlarged right atrium without evidence of right-sided CHF, and 15 mmHg with right-sided CHF [29,30]. Estimated sPAP was calculated by adding estimated right atrial pressure to systolic right ventricular to right atrial pressure gradient. Dogs were categorized as having moderate or severe PH with estimated sPAP of 51—75 mmHg and >75 mmHg, respectively [29]. Eisenmenger physiology was diagnosed if right-to-left intracardiac or extracardiac shunting was documented with agitated saline contrast study. The right pulmonary artery distensibility index was measured as previously described [26]. Briefly, maximum (systolic) and minimum (diastolic) internal diameters of the right branch of the pulmonary artery were measured using a trailing edge to leading edge technique in either long or short axis (the view giving the clearest image was utilized). The right pulmonary artery distensibility index was calculated as follows: ([systolic diameter—diastolic diameter]/systolic diameter) × 100. The pulmonary flow profile was classified as a type I, type II, or type III profile as previously described [3]. Acceleration time was measured from onset of Doppler flow to peak flow velocity. Ejection time was measured from the onset of Doppler pulmonary artery flow signal to the end of signal. An AT:ET ratio was calculated. Indirect blood pressures were measured on days 0 and 15 using an ultrasonic blood flow monitor[f] sphygmomanometer and pneumatic cuff as previously described [31,32].

[f] Model 811-B, Parks Medical Electronics, Aloha, OR.

## Quality of life and voluntary activity

Owners used a previously validated QOL scoring system, functional evaluation of cardiac health (FETCH) questionnaire [33], reflective of 7 days before each assessment (i.e., days 0, 8, and 15). Five possible scores for each question ranged from 1 (poor) to 5 (excellent). Responses for each question were summed to obtain an overall score for each dog at each time point.

A VAS was used to quantitatively measure activity reflective of 24 h (VAS-A) and 7 days (VAS-B) before each assessment. Owners performed a VAS assessment on days 0, 8, and 15. The VAS used a 100-mm scale to assess the range of the owner's perception of clinical sign severity as not affected at all (0 mm) to severe compromise (100 mm) (Supplementary Fig. 1). Distance from 0 to the patient score was measured providing a quantifiable VAS score.

Voluntary activity was measured using a canine-specific activity monitor (AM)[g] that continuously measured magnitude, frequency, and duration of movement that was converted to a raw activity value. This AM was placed ventrally on the neck collar of each dog at baseline evaluation and worn throughout the study duration. Data were downloaded at the second evaluation.

### Adverse events

Short-term AEs occurring throughout the 14 days of dosing were recorded. An AE was defined as any observation, unwanted experience, or reaction that was unfavorable, unintended, and occurred after initiating PDE5I treatment.

## Statistical analysis

Statistical analysis was performed using commercial software.[h] A Shapiro—Wilk test assessed normality. Descriptive continuous data of dogs in each group were summarized using median, Q1, Q3, and range for non-normally distributed data and mean and standard deviation (SD) or standard error for normally distributed data. Continuous baseline descriptive characteristics (i.e., age and weight) were compared using a two-tailed Student's unpaired *t*-test and sex (female/male) with Fisher's exact test. Univariate logistic regression analysis investigated association between treatment and PH group classification with the dependent variable being treatment type

[g] FitBark Inc., Kansas City, MO.
[h] Sigma Plot version 14, Systat Software, San Jose, CA.

**Table 1** Description and comparison of baseline characteristics of the study population.

| Characteristic | Treatment | | *p*-value |
|---|---|---|---|
| | Sildenafil | Tadalafil | |
| Age (years) | 10.5 years (2.9) | 9.1 years (4.9) | 0.44[a] |
| Weight (kilogram) | 6.3 kg (3.2) | 8.1 kg (4.6) | 0.31[a] |
| Sex (FS, FI, MC, MI) | 6, 0, 2, 2 | 7, 0, 3, 1 | 1.00[b] |
| Breed | Mixed breed (n = 2), WHWT (n = 2), Pomeranian (n = 2), YST, Rat terrier, Chihuahua, MP | Chihuahua (n = 2), Shih tzu (n = 2), Border terrier, mixed breed, YST, Standard poodle, MAS, Cairn terrier, WHWT | |

FI, female intact; FS, female spayed; MAS, Miniature Australian shepherd; MC, male castrated; MI, male intact; MP, Miniature poodle; n, number; WHWT, West Highland White Terrier; YST, Yorkshire terrier; SD, standard deviation.
[a] Parametric continuous data presented as mean (SD) and compared with a two-tailed Student's *t*-test.
[b] Categorical data presented as a proportion and compared with Fisher's exact test.

(sildenafil or tadalafil) and the independent variables being groups (i.e. 1—5). Dogs classified with >1 group were categorized as 'group mixed' for this analysis. For between-treatment group comparisons over three time points (i.e. day 0, 8, and 15) of FETCH questionnaire scores and VAS, two-way repeated measures analysis of variance (ANOVA) was performed with post hoc Bonferroni *t*-test making pairwise multiple comparisons. Only dogs with a complete questionnaire and VAS at each time point were included in these analyses. For between-group comparisons of change in voluntary activity during treatment week 1 (i.e. total raw data from days 1—7) with treatment week 2 (total raw data from days 8—14), a two-way repeated measures ANOVA was performed. Change in peak TRFV and estimated sPAP from baseline (before treatment; day 0) to second evaluation (after 14 days of treatment; day 15) was only performed in dogs where these data were available for both time points and were evaluated with a two-way repeated measures ANOVA and with post hoc Bonferroni t-test making pairwise multiple comparisons. Incidence of AEs and frequency of returned residual drug between treatments were compared using Fisher's exact test. Dogs were stratified into four subgroups to assess if differences in the change in peak TRFV from baseline to second evaluation existed between dogs that did or did not have residual drug returned. These subgroups included tadalafil residual returned (A), tadalafil residual not returned (B), sildenafil residual returned (C), and sildenafil residual not returned (D). Two-way repeated measures ANOVA was used for this analysis. The level of significance for all analyses was $p < 0.05$.

## Results

### Descriptive characteristics

Twenty-three dogs were enrolled (Fig. 1). Five were enrolled based on a combination of type III pulmonary flow profiles, RPAD <29%, and/or AT:ET < 0.30, and one based solely on presence of Eisenmenger physiology. Two dogs were excluded for 1 each of the following: death from primary respiratory disease shortly after study commencement and pacemaker lead migration with sick sinus syndrome. The dog that died received 3 doses of tadalafil. Remaining dogs were randomized to sildenafil (n = 10) or tadalafil (n = 11) treatment. Baseline demographics (Table 1) did not significantly differ between treatments. There was no significant association between PH classification group and treatment (Supplementary Table 1).

At least one medication was administered to 62% (13/21) of dogs before enrollment including pimobendan (6), furosemide (5), prednisone (4), hydrocodone (3), enalapril (3), benazepril (3), omeprazole (2), spironolactone (2), and ursodiol (2) and 1 each of the following: aspirin, amlodipine, oclacitinib, carprofen, clopidogrel, diphenhydramine, enrofloxacin, Epakitin, levetiracetam, maropitant, mirtazapine, omega-3 fatty acid, torsemide, subcutaneous fluids, trilostane, aluminum hydroxide, and probiotic. Five dogs had previously been treated with sildenafil and received a 7-day washout before enrollment. One dog required other medications (trimethoprim-sulfa and trazadone) concomitantly with study treatment because of diagnosis of pneumocystis pneumonia.

**Table 2** Comparison of selected hemodynamic and quality of life results at baseline (day 0) in dogs treated with sildenafil or tadalafil.

| Variable | n | Sildenafil | Treatment n | Tadalafil | *p*-value |
|---|---|---|---|---|---|
| Peak TRFV (m/s) | 8 | 3.80 (0.69) | 9 | 4.05 (0.54) | 0.41[a] |
| Estimated sPAP (mmHg) | 8 | 66.90 (20.10) | 9 | 72.90 (20.00) | 0.55[a] |
| PRPV (m/s) | 4 | 2.97 (0.57) | 4 | 3.09 (0.33) | 0.74[a] |
| Type III profiles (yes/total) | 8 | 5/8 | 8 | 4/8 | 1.00[b] |
| AT:ET | 8 | 0.23 (0.19, 0.33) | 8 | 0.24 (0.19, 0.37) | 0.72[c] |
| RPAD (%) | 7 | 9.30 (6.50, 20.50) | 7 | 16.7 (14.70, 18.70) | 0.13[c] |
| FETCH-questionnaire score (0—85) | 10 | 17.60 (13.90) | 11 | 27.0 (21.00) | 0.25[a] |
| VAS-A (0—10 mm) | 10 | 3.60 (1.10,6.60) | 11 | 1.40 (0.30,5.00) | 0.32[c] |
| VAS-B (0—10 mm) | 10 | 3.80 (2.80) | 11 | 3.60 (2.70) | 0.85[a] |

AT:ET, acceleration time (AT) indexed to the ejection time (ET) of pulmonary artery flow; time PRPV, pulmonary regurgitation peak velocity; RPAD, right pulmonary artery dispensability index; FETCH, functional evaluation of cardiac health; m, meter; mmHg, millimeter of mercury; mm, millimeter; n, number; sPAP, systolic pulmonary arterial pressure; s, second; TRFV, tricuspid regurgitant flow velocity; VAS-A, visual analog score 24 h before evaluation period; VAS-B, visual analog score 7 days before evaluation period; SD, standard deviation.

[a] Parametric continuous data presented as mean (SD) and compared with a two-tailed Student's *t*-test.

[b] Categorical data presented as a proportion (yes/total) and compared with Fisher's exact test.

[c] Non-parametric continuous data presented as median (Q1, Q3) and compared with the Mann—Whitney rank sum test.

## Hemodynamics

Comparison of hemodynamic outcome parameters measured at baseline is reported in Table 2. Peak TRFV was available for interrogation, and thus, estimated sPAP at baseline was calculated in 81% (17/21) of dogs. The pulmonic regurgitation peak velocity at baseline was identified in 38% (8/21) of dogs. Forty-three percent (9/21) of dogs had type III pulmonary flow profiles at baseline. Eight dogs had unchanged type III pulmonary flow profiles from baseline to second evaluation. One dog had resolution of notching at second evaluation and another dog had notching first identified at second evaluation. Sixty-seven percent (14/21) and 73% (16/21) of dogs had RPAD and AT:ET measured, respectively, at baseline. There was no significant difference in peak TRFV, estimated sPAP, pulmonic regurgitation peak velocity, frequency of type III pulmonary flow profiles, RPAD, or AT:ET between treatments at baseline (Table 2). Sixty-two percent (13/21) of dogs (sildenafil, n = 5; tadalafil, n = 8) had peak TRFV identified both at baseline and second evaluation. Within this subset of dogs, there was no significant decrease in peak TRFV ($p = 0.056$) or estimated sPAP ($p = 0.06$; Fig. 2) from baseline to second evaluation, irrespective of treatment type. There was no significant difference in estimated sPAP between dogs treated with sildenafil or tadalafil at baseline compared to second evaluation (Fig. 2). While not statistically significant ($p = 0.24$), dogs receiving tadalafil had a greater proportional decrease in peak TRFV (14%) at second evaluation

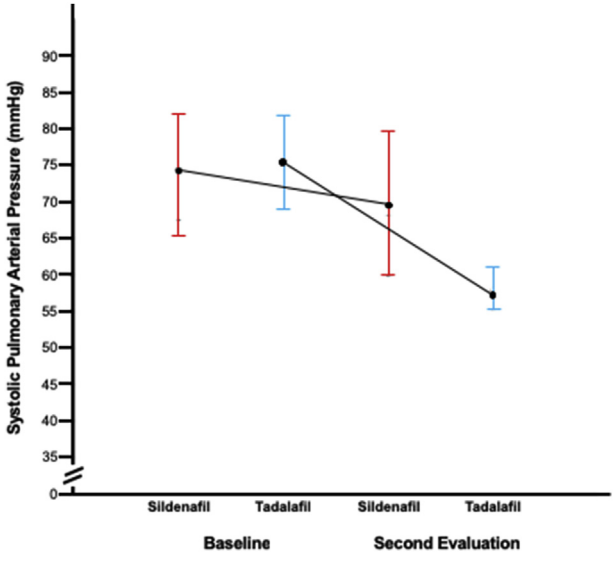


**Fig. 2** Mean (±SE) estimated systolic pulmonary arterial pressure in 13 dogs treated with sildenafil (n = 5, red line) or tadalafil (n = 8, blue line) at baseline (day 0) and at the second evaluation (day 15). The black circles represent the mean and the bars the SE. There was not a significant change in estimated sPAP from baseline to the second evaluation ($p = 0.06$) in dogs treated with a PDE5I (tadalafil or sildenafil) nor was there a significant difference between dogs treated with tadalafil compared to those treated with sildenafil ($p = 0.55$). SE, standard error; sPAP, systolic pulmonary arterial pressure; PDE5I, phosphodiesterase-5 inhibitor.

**Table 3** Comparison of selected hemodynamic results at baseline (day 0) and final evaluation (day 15) in dogs treated with sildenafil or tadalafil in which the echocardiographic parameter was measured at both time points using a two-way repeated measures analysis of variance.

| Variable | Baseline | | | | Final | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Sildenafil | n | Tadalafil | Sildenafil | Tadalafil | A | B | C |
| Peak TRFV (m/s) | 5 | 4.09 (0.58) | 8 | 4.13 (0.52) | 3.94 (0.67) | 3.57 (0.35) | 0.51 | 0.056 | 0.24 |
| Estimated sPAP (mmHg) | 5 | 74.00 (19.63) | 8 | 75.45 (19.69) | 69.67 (22.18) | 57.66 (9.12) | 0.55 | 0.06 | 0.23 |
| PRPV (m/s) | 3 | 2.70 (0.20) | 4 | 3.09 (0.33) | 2.34 (0.47) | 3.15 (0.58) | 0.10 | 0.32 | 0.17 |
| AT:ET | 4 | 0.21 (0.04) | 6 | 0.29 (0.12) | 0.22 (0.06) | 0.27 (0.08) | 0.26 | 0.54 | 0.31 |
| RPAD(%) | 3 | 8.37 (1.66) | 3 | 15.21 (2.72) | 16.40 (7.45) | 23.33 (2.69) | 0.02 | 0.053 | 0.98 |

A, comparison of variable means for treatment, regardless of time; B, comparison of variable means for time, regardless of treatment; C, interaction between treatment and time; AT:ET, acceleration time (AT) indexed to the ejection time (ET) of pulmonary artery flow; time PRPV, pulmonary regurgitation peak velocity; RPAD, right pulmonary artery dispensability index; PRPV, pulmonary regurgitation peak velocity; m, meter; mmHg, millimeter of mercury; mm, millimeter; n, number; sPAP, systolic pulmonary arterial pressure; s, second; TRFV, tricuspid regurgitant flow velocity.

versus dogs receiving sildenafil (4%). Similarly, dogs receiving tadalafil had a greater, yet not statistically significant, decrease ($p = 0.23$) in estimated sPAP (24%) versus dogs receiving sildenafil (6%). A descriptive summary of hemodynamic parameters measured in dogs at baseline and final evaluation are reported in Table 3.

Mean SABP in dogs receiving sildenafil was 143.7 mmHg (SD, 24.7) at baseline and 142.4 mmHg (SD, 27.4) at second evaluation. Mean SABP in dogs receiving tadalafil at baseline was 127.9 mmHg (SD, 22.7) and at second evaluation was 135.7 mmHg (SD, 23.8). There was no significant change in SABP between baseline and second evaluation ($p = 0.61$).

## Quality of life and voluntary activity

The FETCH questionnaire, VAS-A, and VAS-B were available in 95% (20/21) dogs and described descriptively (Table 4). One dog receiving tadalafil was diagnosed with PH while hospitalized; the owner was not in contact with the dog for the entire evaluation period. There was no significant difference in baseline FETCH questionnaire scores or VAS (VAS-A or VAS-B) between treatments (Table 4). A significant decrease (improvement) was noted in FETCH questionnaire scores from baseline to second evaluation ($p = 0.003$), irrespective of treatment type. There was no significant difference in FETCH questionnaire scores between dogs treated with sildenafil or tadalafil at any individual time period. However, dogs receiving tadalafil had a greater proportional improvement in QOL scores (34.9%) from baseline to second evaluation versus dogs receiving sildenafil (25.6%). There was a significant improvement in VAS-B from baseline to second evaluation ($p = 0.024$), regardless of treatment type. A significant difference in VAS (VAS-A or VAS-B) between dogs treated with sildenafil or tadalafil was not identified at any individual time period.

Voluntary activity was measured via AM throughout the 14-day study successfully in 95% (20/21) of dogs. In one dog, technical difficulties precluded data acquisition. Means of total activity accrued over the first 7 treatment days (days 1–7) for dogs receiving sildenafil (n = 11) and tadalafil (n = 10) were 32,549 points (SD, 10,226) and 40,121 points (SD; 17,601), respectively. Means of

**Table 4** Comparison of FETCH questionnaire, VAS-A, and VAS-B scores at baseline (day 0), week 1 (day 8), and at the final evaluation (day 15) in dogs treated with sildenafil (n = 10) and tadalafil (n = 10) using a two-way repeated measures analysis of variance.

| Variable | Baseline | | Week 1 | | Final | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sildenafil | Tadalafil | Sildenafil | Tadalafil | Sildenafil | Tadalafil | A | B | C |
| FETCH questionnaire (0–85) | 17.6 (13.9) | 24.1 (19.7) | 14.2 (13.2) | 18.9 (17.8) | 13.1 (13.8) | 15.7 (18.9) | 0.52 | 0.003 | 0.55 |
| VAS-A (0–10 mm) | 3.96 (2.76) | 2.32 (2.25) | 3.04 (2.17) | 2.40 (2.18) | 2.77 (2.86) | 2.04 (2.14) | 0.29 | 0.31 | 0.52 |
| VAS-B (0–10 mm) | 3.81 (2.82) | 3.49 (2.85) | 3.14 (1.88) | 2.99 (2.03) | 2.87 (2.62) | 2.04 (1.61) | 0.65 | 0.02 | 0.71 |

A, comparison of variable means for treatment, regardless of time; B, comparison of variable means for time, regardless of treatment; C, interaction between treatment and time; FETCH, functional evaluation of cardiac health; mm, millimeter; n, number; VAS-A, visual analog score 24 h before evaluation period; VAS-B, visual analog score 7 days before evaluation period.

total activity accrued over the second 7 treatment days (days 8–14) for dogs receiving sildenafil and tadalafil were 32,168 points (10,809) and 36,651 points (10,962), respectively. There was no significant change in voluntary activity from the first half to the second half of the study period within or between treatments ($p = 0.27$).

### Adverse events

A total of 33% (7/21) of dogs experienced at least one AE during study (tadalafil, n = 5; sildenafil, n = 2). Four dogs receiving tadalafil experienced one AE and one had two AEs: transiently decreased appetite (n = 4), hindlimb weakness (n = 1), and increased sexual behavior (n = 1). Decreased appetite and hindlimb weakness were presumed due to hyperviscosity syndrome from polycythemia secondary to right-to-left patent ductus arteriosus and resolved with therapeutic phlebotomy. The dog that demonstrated augmented sexual behavior was an intact male puppy; the behavior subsided when treatment was switched to sildenafil after the 14-day study period. Two dogs receiving sildenafil experienced mild transient hyporexia not requiring intervention. There was no significant difference between treatment type and incidence of AEs ($p = 0.36$).

### Residual medication

Residual treatment drug was available in 52% (11/21) of dogs (sildenafil, n = 4; tadalafil, n = 7). Mean residual volume from MB in dogs receiving tadalafil was 4.0 mL (SD, 1.8) and from AEB was 12.2 mL (1.6). Mean residual volume from MB in dogs receiving sildenafil was 2.7 mL (1.2) and from AEB was 10.6 mL (2.3). There was no significant association between treatment and frequency of dog owners returning residual drug ($p = 0.395$). There was no significant difference in change of peak TRFV when dogs were stratified into four groups based on treatment and whether they returned residual medication at second evaluation (i.e., tadalafil A, tadalafil B, sildenafil C, and sildenafil D; data not shown).

## Discussion

Collectively, treatment of dogs with moderate to severe PH with PDE5I tadalafil and sildenafil significantly improved QOL measures (FETCH score and VAS) but did not significantly change hemodynamic parameters (peak TRFV and estimated sPAP). Our study failed to show a statistically significant benefit between drugs in peak TRFV, estimated sPAP, subjective measures of QOL, voluntary activity, or incidence of AEs when comparing tadalafil with sildenafil. Previously, both tadalafil [23] and sildenafil [34] improved hemodynamics assessed via right heart catheterization in dogs with experimentally induced PH. Similarly, in people with PH, treatment with either PDE5I resulted in significant improvement in PAP [19,35–43]. However, there are mixed reports on the effect of sildenafil on estimated sPAP measured via transthoracic echocardiography in dogs with naturally occurring PH [8,9,14]. The reason our study did not identify a treatment effect on estimated sPAP is likely multifactorial. One reason could be that Doppler echocardiography was used to indirectly estimate sPAP rather than directly measuring PAP via right heart catheterization. Echocardiographic estimated sPAP measurements are also subject to potential confounding variables like operator skill, overestimation or underestimation of sPAP, and ability of the dog to tolerate echocardiographic examination [30,44,45]. Another potential reason is that these analyses were limited to dogs having the same quantifiable echocardiogram parameter measured at both evaluations. Additionally, the underlying cause of PH was heterogeneous between dogs.

Studies directly comparing efficacy of tadalafil with sildenafil in people with PH are scarce [25,43]. A randomized, non-blinded study in infants with PH and systemic-to-pulmonary shunts did not identify a significant difference in estimated sPAP in 48 h following shunt attenuation or at 1 and 3 months after discharge between infants treated with tadalafil versus sildenafil [43]. Although our study suggests that tadalafil is not superior to sildenafil to decrease estimated sPAP in dogs with naturally occurring moderate to severe PH (i.e., not statistically significant), the percent reduction in estimated sPAP from the start to the end of treatment showed a 24% reduction with tadalafil versus 9% reduction with sildenafil. As suggested by the *a priori* power analysis, our study was likely underpowered. Additional studies with larger numbers of dogs would be beneficial to determine superiority.

There was an overall PDE5 inhibition treatment effect from baseline to second evaluation resulting in improved perceived QOL; however, there was no significant difference between treatments. The decision to compare the efficacy of tadalafil to sildenafil rather than placebo was made because all dogs had moderate to severe PH and withholding treatment would have been unethical. No dogs that underwent a washout period developed adverse clinical signs before enrollment. To the

authors' knowledge, no studies directly compare QOL in people or dogs with PH treated with tadalafil versus sildenafil. However, one study compared QOL in people with PH before and after conversion from sildenafil to tadalafil [20]. That study utilized a treatment satisfaction questionnaire for medication and found that a greater percentage of people were satisfied (55%) than dissatisfied (19%) with conversion to tadalafil [20]. Another study identified an improvement in QOL and convenience (i.e., less frequent dosing) after conversion from sildenafil to tadalafil with 83% (15/18) of participants electing to continue tadalafil after study conclusion [43].

Our study did not identify a within-treatment difference in QOL. This is in contrast to trials in people demonstrating a significant improvement in QOL when treated with sildenafil or tadalafil compared to placebo [18,19]. In dogs, a randomized, double-blinded, placebo-controlled clinical trial identified significant improvement in QOL after 4 weeks of sildenafil treatment in dogs with PH secondary to chronic valvular disease [14]. One possible reason for our contrasting results with the aforementioned study could be that our 2-week study period was not long enough to identify a significant difference. Also, our cohort included dogs with a different disease etiologies leading to PH. Our heterogeneous population could have masked significant effects seen in individual disease classifications. A small sample size in each group could have contributed to failure to attain statistical significance. Future studies evaluating effects of sildenafil and tadalafil on a larger number of dogs over longer periods of time and/or with more uniform PH disease classifications are warranted.

Exercise capacity in people with PH is commonly evaluated through total distance walked in 6 min [18,19]. Safety measures are incorporated into 6-min walk distance tests to mitigate risks related to overexertion (e.g., syncope, respiratory distress). These measures include availability of prearranged rest areas and rely heavily on a person's acknowledgment and verbalization that rest is needed. Our study utilized AMs to track voluntary activity at home in lieu of a 6-min walk distance test because of the inherent risk associated with forced exercise in dogs with moderate to severe PH. No significant difference in voluntary activity was noted between the first and second weeks of treatment. These results contrast with a previous crossover study in dogs with PH utilizing a similar AM demonstrating a significant increase in voluntary activity when receiving sildenafil versus placebo [14]. However, results from that study were likely skewed because only 60% (8/13) of dogs completed both phases and dogs were more likely to be withdrawn during placebo treatment [14]. Furthermore, that study only included dogs with PH as a result of chronic valvular disease, whereas our present study included dogs with a large spectrum of diseases. Dogs with severe concurrent diseases, especially affecting lung function, could have less voluntary activity, irrespective of PH, than dogs with only chronic valvular disease.

Frequency of AEs with tadalafil and sildenafil in our study was not significantly different. This agrees with a recent meta-analysis evaluating safety of oral PDE5I in people showing AEs after administration of tadalafil or sildenafil occur at a similar frequency and severity [46]. Interestingly, while safety profiles for sildenafil and tadalafil in people are similar, there can be intraclass variability in type and severity of AEs. One study reported that 46% of people with PH who had experienced intolerable AEs while taking sildenafil were able to tolerate and transition to tadalafil without loss of clinical efficacy [47]. Thus, tadalafil could represent a useful alternative in dogs with PH if intolerable AEs occur with sildenafil.

Adverse events were reported with both treatments in our study with transient loss of appetite being most commonly reported. Most frequent AEs reported in people administered with PDE5I include transient headaches, myalgia, dyspepsia, and flushing [47]. Reported AEs in dogs with PH administered sildenafil varies presumably because of wide spectrum of underlying disease etiologies, dosages, and study designs. Reported AEs in sildenafil-treated dogs with PH include lethargy, somnolence, nasal discharge, erect ears, gastrointestinal effects, and cutaneous flushing [8,9]. Overall, tadalafil and sildenafil seem to be well tolerated in dogs with PH. Additional studies evaluating varied dosages and longer durations are needed to understand the safety of tadalafil in dogs.

This pilot study had several limitations. A significant between-treatment difference was not identified possibly because our study was underpowered. A power calculation to estimating sample size *a priori* was performed, indicating 356 dogs would be needed to attain significant difference [25]. As this would not be easily achievable, the goal was to compare efficacy of tadalafil with sildenafil in dogs with PH, and if a significant difference was not identified, pilot data would be secured for future studies. *Post hoc* analysis of our results revealed 55 dogs would be needed to achieve $\beta = 0.20$ and $\alpha = 0.05$ and detect a significant difference in estimated sPAP. Additionally, the study period was purposefully short to mitigate dropout, maximize owner compliance, and limit exposure to tadalafil in case it lacked clinical

efficacy. Clinical efficacy and hemodynamic improvement in dogs with PH administered sildenafil occurs within 2 weeks after initiation [9,48]. Inclusion of a placebo group could have been shown if the 'natural course' of PH would have led to significant declines in hemodynamics, QOL, and activity that could have been stabilized with PDE5I. Additionally, echocardiographic studies were performed and measured by three different investigators and interobserver and intraobserver variability were not assessed. This study was subjected to owner bias in which use of a positive control could bias owner scores after treatment compared to baseline. Dogs were mainly enrolled based on presence of TR supporting moderate to severe PH; however, in absence of TR or when optimal alignment was not obtained, a combination of $\geq$2 of type III pulmonary flow profiles, RPAD $<$29.5%, or AT:ET $<$ 0.30 were used as a surrogate. All dogs were administered sildenafil or tadalafil compounded into a suspension for masking purposes. It is not known if the compounded formulation impacted pharmacokinetics. Future pharmacokinetic studies are needed to compare commercial and compounded formulations. At least one medication was administered to 62% (13/21) of dogs before enrollment, which could have potentially impacted QOL assessment. However, concurrent therapy for a comorbid condition was allowed only if initiated $>$1 week before enrollment as to minimize this possible effect. The one dog with pneumocystis pneumonia started on novel medication concomitantly with the study drug at enrollment was removed from statistical analyses evaluating QOL parameters.

It is possible that a significant difference could have been identified if a greater dosage of tadalafil or sildenafil had been used. The dosage of tadalafil (i.e. 2 mg/kg) is the minimum effective oral dosage to significantly decrease PAP in dogs with experimentally induced PH [23]. To allow relatively equal clinical comparison of tadalafil and sildenafil, the lowest clinically utilized effective dose of sildenafil (i.e. 0.5 mg/kg) was used. In addition, sildenafil administered at 0.5 mg/kg once every 8 h [48] and once every 12 h [49] has been reported to be effective in some dogs with PH. Ensuring compliance with administration of medication is also critical for determining efficacy of therapy. Residual medication was returned in only 50% of study dogs; however, there was no significant association between treatment and frequency of residual drug being returned or in change of peak TRFV between dogs that did and did not have residual drug returned.

## Conclusions

In conclusion, our pilot study indicated that administration of tadalafil or sildenafil to dogs with moderate to severe PH resulted in improvements in QOL and VAS-B scores from baseline to the second evaluation. However, tadalafil was not shown to be superior to sildenafil; this will require future studies with larger numbers of dogs. Both PDE5I were generally well tolerated. Tadalafil could represent a viable alternative to sildenafil for dogs that experience intolerable AEs or in which once daily dosing is desired.

## Conflict of Interest Statement

Eli Lilly generously donated tadalafil for use in this clinical trial but had no association with study design, data collection, data analysis, or manuscript draft preparation. None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the article.

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## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvc.2019.05.001.

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