EFFICACY OF LIQUID SILDENAFIL AS A NOVEL TREATMENT FOR CANINE GENERALIZED MEGAESOPHAGUS: A RANDOMIZED CROSSOVER STUDY

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of SUSAN OLIVIA MEHAIN find it satisfactory and recommend that it be accepted.

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Abstract

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Megaesophagus (ME) is characterized by dysmotility and dilation of the esophagus, causing regurgitation and carrying a poor prognosis. If it can be delivered to the stomach of dogs with ME, sildenafil may cause short duration relaxation of the gastroesophageal sphincter thus improving clinical signs in dogs with ME via increased esophageal clearance. The objectives of this study were to determine if liquid sildenafil could be delivered to the stomachs of dogs with ME and have significant effects on esophageal clearance, frequency of regurgitation, body weight, and perceived quality of life compared to no treatment or a placebo. In this blinded, randomized, crossover study, 10 healthy, client-owned dogs, previously diagnosed with ME received either sildenafil (1 mg/kg per os [PO] q12h) or a placebo for 14 days, followed by a 7-day washout, then the opposite treatment for 14 days. Esophageal clearance time was assessed prior to treatment (baseline), and on day 1 of each treatment period using videofluoroscopy performed over 30 minutes with dogs in an upright position. Owners kept logs of regurgitation episodes for 2 weeks before, during the treatment periods, and during the washout periods. Clearance of liquid was variable in dogs; liquid was administered a total of 30 times. It cleared prior to slurry ingestion, moved into the stomach following slurry ingestion, or did not clear in

five, sixteen, and nine times, respectively. There were no significant differences in regurgitation episodes between untreated, placebo, or washout periods; quality of life scores between no treatment, sildenafil, or placebo; body weight after placebo; or esophageal clearance between untreated, placebo or sildenafil treatment. Sildenafil did result in significant reductions in regurgitation episodes ($p < 0.05$) and increased body weight ($p < 0.05$) compared to no treatment and placebo. Results indicate there is potential for improved management of dogs with ME treated with long-term liquid sildenafil.

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Dedication

I would like to dedicate this thesis to my parents.

CHAPTER ONE: INTRODUCTION

Megaesophagus (ME) is a disorder characterized by reduced to absent esophageal motility, resulting in dilation and accumulation of ingesta within the esophageal lumen; it is the most common cause of regurgitation in dogs (Marks 2017; Washabau 2003; Harvey et al., 1974). Canine ME can be congenital or acquired; the acquired form may occur secondary to a multitude of etiologies, including myasthenia gravis, hypoadrenocorticism, hypothyroidism, polymyopathies, and dysautonomia, but is most often idiopathic (Gaynor et al., 1997; Harvey et al., 1974; Jaggy et al., 1994; McBrearty et al., 2011; Washabau, 2003). In dogs affected by generalized ME, complications such as esophagitis, weight loss, malnourishment, dehydration, and aspiration pneumonia are commonplace and lead to a guarded to poor prognosis, regardless of the underlying cause (Marks 2017; Washabau 2003; Harvey et al., 1974; Glidewell 1983; Guilford et al., 1996; McBrearty et al., 2011; Boudrieau et al., 1985; Simpson 1994). Previous studies have estimated survival times of 1-3 months after diagnosis with an overall case mortality rate of 74% (Washabau 2003; McBrearty et al., 2011). Death or euthanasia is often the result of severe complications or owner frustrations with ME management, which is limited, life-long, and primarily focuses on feeding strategies to reduce retention of ingesta and subsequent regurgitation (feeding upright for prolonged periods, experimenting with different food consistencies) (Harvey et al., 1974; Simpson 1994). In order to improve management, an effective treatment to reduce food and liquid retention in the esophagus would be ideal to reduce regurgitation and development of life-threatening complications. Further investigation into medical management options is critical to improving the quality of life and survival of dogs with ME.

In normal dogs, swallowing and then distension of the esophageal lumen by the food bolus stimulates esophageal peristalsis. The lower esophageal sphincter (LES) then relaxes, allowing it to pass into the stomach (Marks 2017; Guilford et al., 1996; Watrous et al., 1979; Hershcovici et al., 2011). Historically, goals of treatment have been to stimulate esophageal motility. Prokinetics aimed at increasing peristalsis by stimulating smooth muscle (ex. metoclopramide, cisapride) have been used in an attempt to increase esophageal motility; however, they are ineffective in the canine esophagus, which is composed of striated muscle, and may have detrimental effects by tightening the smooth muscle of the lower esophageal sphincter (LES) (Harvey et al., 1974; Kempf et al., 2014; Ullal et al., 2016).

In humans, achalasia is a condition in which the LES fails to open and there is esophageal hypomotility. This causes similar clinical signs to those seen in dogs with ME. Treatment options for achalasia typically target relaxation or opening of the LES, and include botulinum toxin injection, self-expanding metal stents, surgical or endoscopic myotomy, and esophageal dilation (Furuzawa-Carballeda et al., 2016; Pandolfino et al., 2015; Tuason et al., 2017). Pharmacologic intervention has also been investigated using sildenafil, a phosphodiesterase-5 inhibitor that relaxes smooth muscle, including the LES in humans and cats (Bortolotti et al., 2000; Bortolotti et al., 2001; Bortolotti et al., 2002; Rhee et al., 2001; Moreland et al., 1998; Eherer et al., 2002; Zhang et al., 2001). In veterinary medicine, sildenafil is used for the treatment of pulmonary hypertension as it causes relaxation of the pulmonary vasculature (Kellum et al., 2007; Brown et al., 2010; Murphy et al., 2017; Kellihan et al., 2012). In a previous study on pharmacokinetics of sildenafil in dogs, a dose of 1 mg/kg was found to reach therapeutic levels with no significant adverse effects noted (Nichols et al., 2002). A recent study evaluated the effects of 1 mg/kg of liquid sildenafil in young dogs diagnosed with congenital megaesophagus. The study used

radiographs to try to assess response to treatment based on esophageal dilation and also evaluated regurgitation frequency. In addition, *in vitro* evaluation of dissected LESs from dogs not affected by ME were evaluated for relaxation associated with sildenafil exposure (Quintavalla et al., 2017). This study revealed that liquid sildenafil is well tolerated at a dose of 1 mg/kg and there was clinical improvement based on number of regurgitation episodes recorded by owners. Dissected LESs from normal dogs had reduced basal tone and increased electrically reduced relaxation. However, this study did not take into consideration the ability for the esophagus to continue maturing up to 1 year of age, assess for evidence of LES relaxation in dogs with ME, or assess real-time effects of the drug on the movement of ingested material (Harvey et al., 1974; Diamant et al., 1974; Bexfield et al., 2006).

No studies to date have assessed for evidence of LES relaxation in dogs with ME in response to sildenafil administration, or the real-time effects of the drug on the movement of ingested material. A common method used to assess swallowing, esophageal function, and esophageal clearance is contrast videofluoroscopy (VF). Real-time esophageal motility and esophageal clearance can be observed, and patients do not typically require previous training or sedation, making it a safer choice for dogs with ME or dysphagia (Pollard et al., 2016; Bonadio et al., 2009; Haines et al., 2019).

The purpose of this study was to assess the potential for liquid sildenafil to reduce severity of clinical signs and reduce complications associated with ME. Specific aims were to determine if liquid sildenafil was able to reach the stomach in a population of dogs with ME; to compare transit times of liquid and solid foods in the same dog with and without prior administration of liquid sildenafil; and to record and compare frequency of regurgitation and

owner perception of quality of life without liquid sildenafil and during a period of time receiving liquid sildenafil at home.

CHAPTER TWO: METHODOLOGY

Animals

A total of 10 client-owned dogs over 1 year of age that were previously diagnosed with megaesophagus (ME) and had stable disease defined as having no new clinical signs reported by owners within 3 months prior to enrollment, were recruited on a volunteer basis with informed owner consent. Prior to enrollment, work-up for ME included a total T4, baseline cortisol with ACTH-stimulation testing if indicated, creatine kinase, and acetylcholine receptor antibodies. Health status was then confirmed on enrollment by review of medical records, physical examination, complete blood count, biochemistry panel, urinalysis, and thoracic radiographs to screen for evidence of aspiration pneumonia. Dogs were excluded from the study if they were diagnosed with concurrent diseases not directly associated with their ME diagnosis; had received medications that could interfere with the function of the LES (i.e. metoclopramide, cisapride, sildenafil) within the 3 weeks prior to enrollment; had evidence of aspiration pneumonia on screening radiographs; or would not tolerate handling/sitting upright in a Bailey chair for a prolonged period of time. The study's enrollment of dogs was approved by the Washington State University Institutional Animal Care and Use Committee.

Study Design

In this blinded, randomized crossover study, dogs were randomly assigned to receive either sildenafil (compounded by a commercial pharmacy[a] to a strength of 20 mg/ml using almond oil as the carrier solution) or a placebo for a total of 14 days, followed by a minimum washout period of 7 days. The placebo was the carrier solution of the liquid sildenafil, without the addition of the active sildenafil. After this period, dogs were then switched to the opposite treatment group (sildenafil or placebo) for a total of 14 days. All dogs received 1 mg/kg of

compounded sildenafil by mouth every 12 hours (Quintavalla et al., 2017) or an equivalent volume of placebo. Dogs were evaluated on three separate days as detailed below.

Prior to the initial visit, owners kept a 2-week log of their dog's regurgitation episodes. During the first visit information was collected from the owners detailing management and historical points, such as the typical frequency, timing, and nature of the regurgitation; feeding strategies; ability to tolerate water; current diet (including consistency of the food); and other medical history, including any prior episodes of aspiration pneumonia.

Videofluoroscopic Imaging

Videofluoroscopy was performed with dogs sitting upright in a Bailey chair using the same fluoroscopic unit (OEC 9600 C-Arm Unit, GE Healthcare, Salt Lake City, UT) for all dogs. Videofluoroscopic images of 5-10 seconds were taken at the time of administration of liquid or slurried food and then at 5-minute intervals (see below).

Day 1. Following health status determination, baseline imaging with the fluoroscopy unit was performed. For baseline imaging, dogs were seated upright in a Bailey chair and received 5 mL of a 25% iohexol solution diluted with broth. Videofluoroscopic images were taken as described above until all liquid was cleared, or to a maximum of 30 minutes post-administration. The dog then received a slurry of canned food, water, and iohexol (20mL iohexol per 1 cup food). Videofluoroscopic images were again obtained as described above until all slurry passed or 30 minutes was reached, whichever occurred first. In all cases, the amount of food was calculated as 10% of the individual dog's RER (calculated as [30 x body weight in kilograms] + 70), so as to standardize volumes for the variably sized dogs.

Day 2. On the second day of the first visit, dogs were randomized into the treatment group or placebo group. The pharmacist drew up the dose of treatment/placebo for in-hospital

use. The researchers and the owners were blinded to which group each dog was in. Dogs received either sildenafil (20 mg/mL) at a dose of 1 mg/kg,[26,27,30] or placebo liquid diluted to a total volume of 5 mL with liquid iohexol and broth (25% concentration). After administration of the liquid, imaging was performed as described above. Once 5 minutes was reached or all liquid cleared, slurry was given, and VF performed again. Dogs were then discharged with the placebo or treatment, depending on the group they were assigned too. The owners kept a journal of regurgitation characteristics (frequency, volume, consistency, etc.) at home for a 3-week period; dogs were medicated/received placebo at the time of feeding for the first 2 weeks, and then had administration discontinued for 1 week. To allow for drug washout, after a minimum of 1 week without administration of either drug or placebo, the dog returned for a second visit.

Day 3. At the second visit, dogs were given the alternative treatment to what they received at visit 1. Videofluoroscopic imaging was performed as described above. Dogs were then given the placebo or drug for an additional 2 weeks, followed by 1 week with no medication administration; owners recorded regurgitation episodes for the full 3-week period. At the completion of each treatment, a weight was collected on the dog.

Quality of life assessment and treatment prediction

Owners were asked to provide an assessment of their dog's quality of life prior to enrollment in this research study and following each treatment (sildenafil and placebo). Quality of life assessment was based on parameters such as attitude, energy level, and general perception of ability to perform their normal daily activities. At the end of the study, blinded owners were asked to predict during which treatment period they thought their dog was receiving sildenafil or the placebo.

Statistical Analysis

In order to determine an adequate number of dogs to enroll, data was used from previous research (Haines et al., 2019). With a power of 80% and alpha level of 0.05 to detect at least a 5-minute difference between placebo vs. sildenafil esophageal clearance, a sample size of 8 dogs was required.

Statistics were performed using a commercially available statistical software package (IBM SPSS®). Descriptive statistics were calculated and are reported as mean (+/- standard deviation), with inclusion of range where clinically relevant. Wilcoxon signed rank test for paired data was used to compare pre-treatment and post-treatment values, and placebo versus treatment effects. Non-parametric tests were used to account for non-normal data and sampling distribution, which is expected in this study design. Frequency histograms were used to confirm non-normality, as well as skewness and kurtosis statistics. Homoscedasticity was not calculated due to non-normality of data and variances. Quality of life data were numerically coded and reported using descriptive statistics. Missing data were handled with case-wise exclusion, where relevant. Correlation between body weight and number of regurgitation episodes was handled with a linear regression analysis model. A priori alpha level (p-value) was set at a significance of alpha ≤ 0.05.

CHAPTER THREE: RESULTS

Animals

A total of ten dogs completed the study. There were four spayed females and 6 neutered males. Breeds included four German shepherds, one Labrador retriever, one West highland white terrier, and four mixed breeds. The mean weight of dogs at the start of the study period was 21.6 kg (range 5.1 kg to 40.1 kg) and the median age of enrolled dogs was 3 years (range 1 year to 8.8 years). Three dogs received a diagnosis of congenital ME and the remaining 7 dogs were diagnosed as idiopathic acquired ME. No animals showed any adverse effects while receiving sildenafil or the placebo.

Regurgitation episodes

The mean number of regurgitation episodes per week was 8 (+/- 6.9; 1.5 – 19.5) at baseline. A standard 7-day washout period was imposed on each patient between treatment and placebo or placebo and treatment, as described in the study methodology. After two weeks of placebo, the mean number of regurgitation episodes was 7.5 (+/- 9; 0 - 28). After two weeks of sildenafil, the mean number of regurgitation episodes was 5 (+/- 5; 0 - 14.5). There was no statistically significant difference between the number of regurgitation episodes per week at baseline and the number of regurgitation episodes per week after 2 weeks of placebo. There was a statistically significant difference between regurgitation episodes per week at baseline and regurgitation episodes per week after 2 weeks of sildenafil ($p = 0.05$). There was no difference between any of the groups after the washout period. See Table 3.1.

Body weight

The difference between body weight at baseline and body weight after placebo was not statistically significant. However, the difference between body weight at baseline and body weight after sildenafil was statistically significant ($p < 0.05$). See Table 3.1.

Table 3.1 Summary of regurgitation episodes per week, quality of life, and body weights for dogs before treatment, receiving placebo or sildenafil, and during washout periods post-placebo and post-sildenafil.

		N	Minimum	Maximum	Mean	Std. Deviation
Regurgitation episodes/week	Baseline	10	1.50	19.50	8.00	6.94
	With placebo	10	.00	28.00	7.50	8.97
	Post-placebo	10	.00	17.00	4.70	5.36
	With sildenafil	10	.00	14.50	5.05	5.11
	Post-sildenafil	10	.00	12.00	5.40	5.15
Quality of life	Baseline	10	1.0	2.0	1.65	.41
	With placebo	10	1.0	2.0	1.60	.52
	With sildenafil	10	1.0	2.0	1.65	.47
	Post-placebo	10	-1	2	.00	.94
	Post-sildenafil	10	-1	1	.00	.67
Body weight	Baseline	10	5.10	40.10	21.38	8.78
	Post-placebo	9	5.80	25.90	20.10	6.20
	Post-sildenafil	9	6.00	41.30	22.38	9.26

Clearance time

The mean time of clearance of the liquid based on videofluoroscopic imaging (in increments of 5 minutes) was 22 minutes (+/- 11 minutes) at baseline. Of the 30 VF episodes (3 episodes per dog) where liquid was administered followed by the meal slurry, 5 episodes showed movement of the liquid into the stomach without subsequent slurry meal administration. Conversely, 16 episodes occurred where the liquid was initially stagnant in the esophagus, but subsequent administration of a slurry meal resulted in most or all of the liquid being moved into the stomach. In the remaining 9 episodes, liquid did not move into the stomach, or simply mixed with the slurry, despite subsequent administration of a meal slurry using the same protocol as above. Of the nine episodes where the liquid did not move into the stomach despite subsequent

slurry administration, 6 of them occurred in just two dogs, with the three remaining episodes occurring in an additional two dogs.

The mean clearance time of the slurry at baseline was 21 minutes (+/- 10.5 minutes). The mean clearance time of the slurry after placebo was 22.5 minutes (+/- 8.9 minutes). The mean clearance time of the slurry after sildenafil was 21 minutes (+/- 9.4). There were no differences between time of clearance of the slurry after placebo from baseline or after sildenafil treatment using VF measurements.

Quality of life

Quality of life was assessed by owners as good, good to excellent, or excellent in all cases. There was no difference in quality of life scores between any of the groups. Importantly, quality of life was not perceived as worse in the treatment group. Change in quality of life averaged unchanged in both the sildenafil and placebo groups. There was no difference between the change in quality of life between sildenafil and placebo. There was no significant relationship between body weight and number of regurgitation episodes in either the treatment or placebo group in the linear regression analysis model.

Owner prediction of placebo vs. sildenafil

Five owners correctly predicted when their dog was in the treatment group compared to placebo; two owners could not distinguish a difference and three owners guessed incorrectly.

Outliers

Outliers were identified using standardized z-scores, with a z-score cutoff of +/- 1.96 (representing 95% of the data). Only 5 outliers were detected in all the data. Two were in case 6 with number of regurgitation episodes after two weeks on placebo and after wash-out with z-scores of 2.29 and 2.3, respectively. The other two were in case number 7, with a z-score of 2.13

for weight at baseline and 2.04 for weight after sildenafil. The final outlier was in case 9, with weight after placebo, which was -2.3. Because the data were non-normally distributed, and because of the type of analysis used, these outliers are relatively arbitrary and only used to check assumptions but are not relevant in the final model.

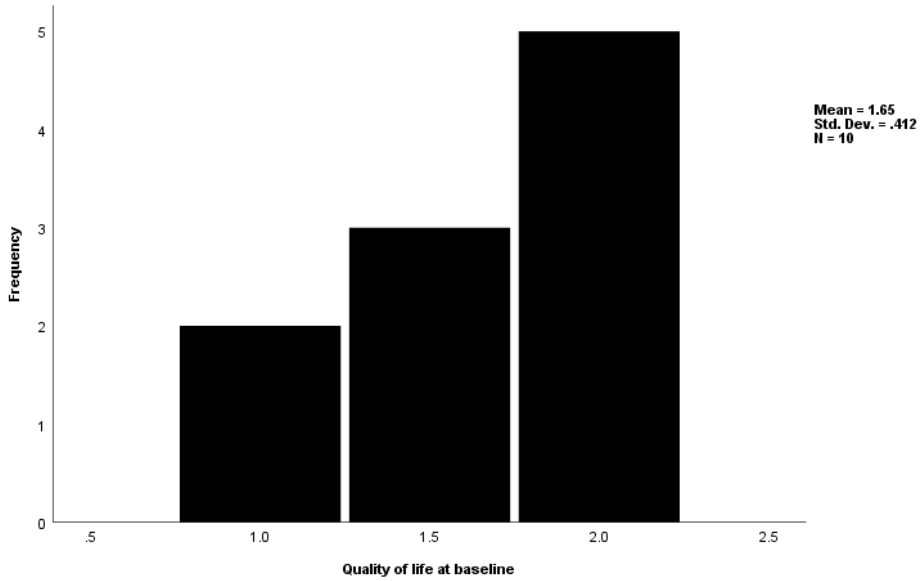

Figure 3.1 Owner-perceived quality of life of 10 dogs with megaesophagus at baseline, prior to treatment with sildenafil or a placebo.

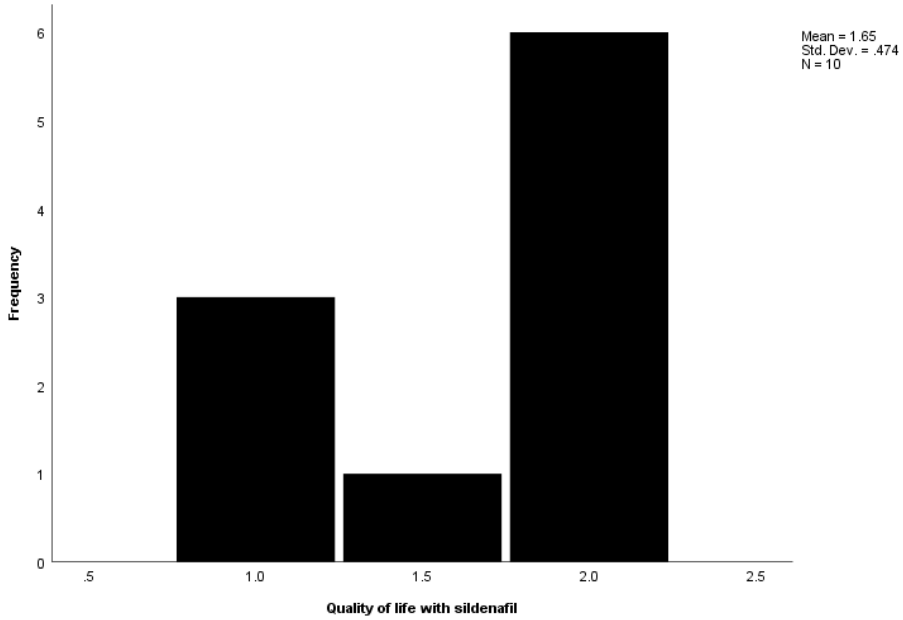

Figure 3.2 Owner-perceived quality of life of 10 dogs with megaesophagus while receiving treatment with sildenafil.

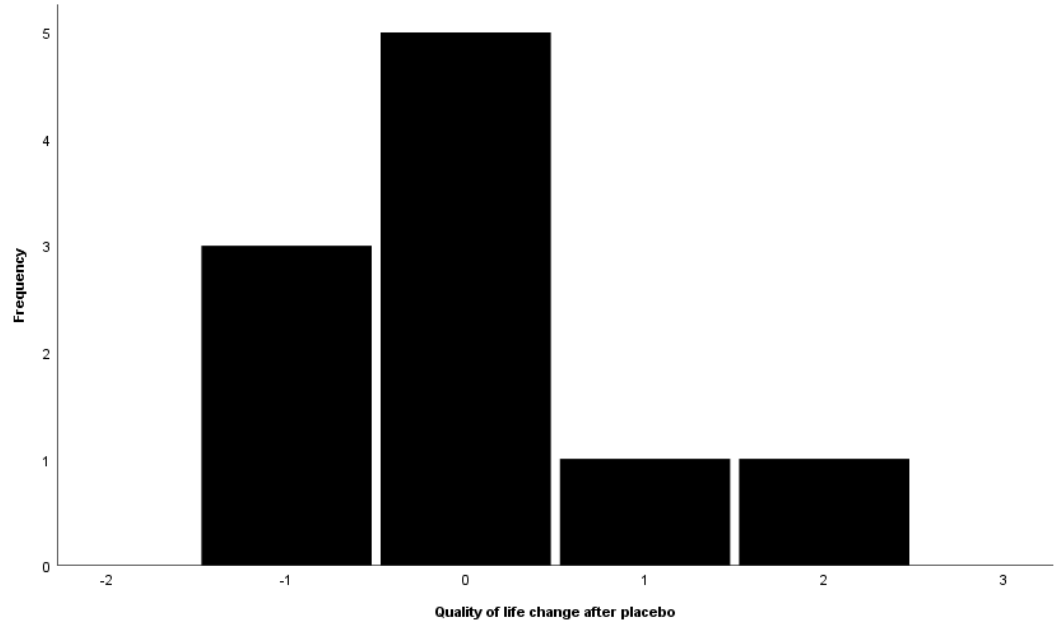

Figure 3.3 Change in quality of life score in 10 dogs with megaesophagus following 14 days of placebo administration.

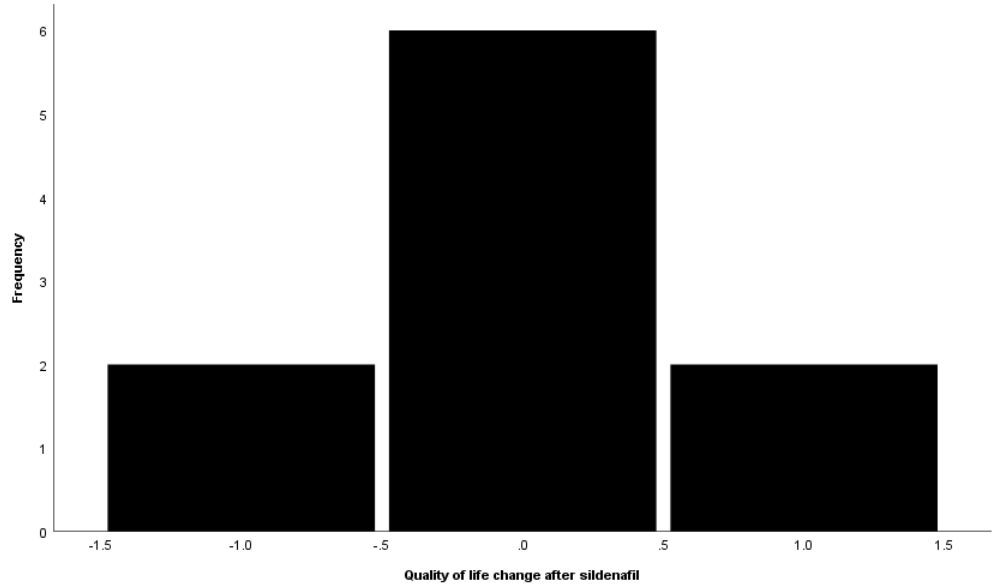

Figure 3.4 Change in quality of life score in 10 dogs with megaesophagus following 14 days of sildenafil administration.

CHAPTER FOUR: DISCUSSION

The results of this study found that dogs taking sildenafil had significantly fewer episodes of regurgitation compared to baseline or when taking a placebo. There were no significant changes in owner-perceived quality of life or difference in videofluoroscopic clearance time of meals. In some dogs, liquid sildenafil did not reach the stomach prior to feeding. These results suggest that sildenafil could be beneficial in reducing the number of regurgitation episodes experienced by dogs with ME, but efficacy may vary significantly between individual dogs.

Due to frequent regurgitation, malnourishment and poor body condition may be common complications of ME. It follows that if regurgitation episodes are reduced, more food reaches the stomach and subsequent weight gain would be expected. In this study, weights were obtained at baseline and at the end of each treatment period and found a significant increase in body following sildenafil treatment but not placebo. This suggests that sildenafil may reduce regurgitation events or volume of regurgitant enough to allow for weight gain, which is an important finding given that chronic malnutrition is a reason why some owners elect euthanasia in dogs with ME.

Interestingly, in spite of reduced regurgitation episodes and increased body weight while on sildenafil suggesting a positive clinical response to therapy, no difference was found on VF between clearance time at baseline, with sildenafil or with placebo. Several factors could account for this, including failure of the liquid sildenafil to pass into the stomach with enough time to become effective within the measured time. Clearance of liquid was variable and in most dogs liquid did not pass prior to feeding slurry. Once slurry was given, the liquid then moved into the stomach in a number of dogs. Of the nine episodes where the liquid did not move into the stomach prior to or in spite of subsequent slurry administration, 6 of them occurred in two dogs. This suggests there may be a patient-dependent component to LES relaxation and ability of a

meal slurry to effectively initiate esophageal transit of an administered liquid. It may also be associated with individual patient retention of other liquids (saliva and/or water) in the esophagus between meals, which may cause the liquid to get hung up above the LES and prevent passage into the stomach. In addition to variable transit time through the esophagus, absorption may be variable in individual dogs, and the 30 minutes during which VF was performed may not have been sufficient time to allow for absorption and onset of action of the medication. Sildenafil is well tolerated in dogs, in which the half-life is 3 to 5 hours (Akabane et al., 2018; Walker et al., 1999). In addition, giving sildenafil with a meal does not significantly affect the absorption, maximum concentration, or half-life, which is in accordance with findings in humans and important when considering administration to dogs with ME (Akabane et al., 2018; Nichols et al., 2002). Studies assessing its effect on LES relaxation in people have shown the onset of action to range from 10 – 20 minutes after administration, and duration of effect to last approximately 1 hour (Bortolotti et al., 2000; Bortolotti et al., 2001; Bortolotti et al., 2002; Rhee et al., 2001). This is not ideal as a long-term treatment for people but would be sufficient for dogs at times of meal feeding. In addition, there does not appear to be a lasting effect of sildenafil given that there was no significant difference in the number of regurgitation episodes during the washout period as compared to baseline. This is an important finding as prolonged relaxation of the LES could in fact be detrimental if it increased the incidence of reflux. Although perhaps not practical for use in people with disorders of the LES, it is ideal to have a short-acting medication that can be used when meal-feeding dogs.

As mentioned above, in some dogs the liquid remained in the fluid within the dilated esophagus, and then mixed into the slurry. This might have prevented passage of a sufficient dose into the stomach, potentially resulting in dose variability. Dogs with ME have a wide

variation in clinical severity of their disease. In this study, some dogs did show a marked difference between sildenafil and placebo both clinically and on VF, but this was not reflected in the group findings. This may indicate that sildenafil will prove useful based on the individual response, but a larger population of dogs would be needed to determine if there is a clear group effect.

One aim of this study was to determine if liquid sildenafil could be delivered successfully to the stomach of dogs with ME. Liquid medication was chosen based on a previous study which found that liquids did not clear well in the majority of dogs with ME unless followed by a meal (Haines et al., 2019). Based on the finding in this study that many of the dogs who did not clear the liquid at baseline did still clear it following administration of slurry, all owners were instructed to give the medication in an upright position and then feed their dogs shortly (2-5 minutes) after giving the medication. Alternatively, capsules or tablets could have been used but this may have led to inconsistent dosing due to entrapment in the esophagus and could result in drug overdose. However, this was not evaluated in the current study.

Quality of life at baseline was assessed by owners as good, good to excellent, or excellent in all cases. Change in quality of life averaged unchanged in both the sildenafil and placebo groups and there was no difference between the change in quality of life between sildenafil and placebo, which was not consistent with the only other study that has evaluated the effect of sildenafil on dogs with ME. Although that study did not evaluate real-time clearance of ingested material, there was clinical improvement based on number of regurgitation episodes recorded by owners (Quintavalla et al., 2017). Additionally, only half of the owners correctly predicted when their dog was on sildenafil compared to the placebo. This could be a reflection of a true clinical difference in individual dogs, or random chance given that three owners guessed wrong and two

could not detect a difference when asked which treatment they felt contained the active ingredient. One consideration is that many dogs were already rated as having good to excellent quality of life at baseline, leaving little room for improvement. In dogs mildly affected by their ME, small changes will be difficult to detect. Animals with severe disease could potentially show marked improvement, but alternatively the liquid medication might not consistently reach the stomach resulting in failure to detect a difference. Moderately affected individuals could be the group to show the most difference due to ability for medication to reach the stomach for absorption and a detectable difference in clinical signs to be appreciated. Selection and screening for enrollment in the current study did not exclude dogs based on severity of their disease; however, they had to have presented with regurgitation as part of their clinical signs in order to detect a response to therapy.

Importantly, quality of life was not perceived as worse in the treatment group. Our study found that sildenafil was well tolerated at a dose of 1 mg/kg every 12 hours, and none of the dogs had adverse effects when receiving either the placebo or sildenafil. This is consistent with findings in other studies using the same dose of sildenafil in dogs with no reported adverse effects (Brown et al., 2010; Kellum et al., 2007; Quintavalla et al., 2017). This is especially important in this patient group, as dogs with ME are already at increased risk of regurgitation, aspiration pneumonia, and malnutrition, so medications that cause nausea, vomiting, or reduced appetite can be especially detrimental to their quality of life and health. In addition, a previous study reported that giving sildenafil with a meal did not significantly affect the absorption, maximum concentration, or half-life, which is relevant in dogs with ME who will likely receive the medication along with a meal. A possible downside of liquid sildenafil is the potential for changes in absorption, bioavailability, and efficacy due to the compounding process.

Our study has several limitations, including the small sample size. Future studies enrolling a larger number of dogs would be beneficial to confirm the effect found in our study. The study relied heavily on owner compliance, as they had to medicate their dogs and record regurgitation habits for a total of 2 months. However, logs of the owners of enrolled dogs that completed the study were indicative of excellent compliance. In addition, recording characteristics of the pet's regurgitation was subjective. Although this subjectivity could not be eliminated, to try to reduce bias, owners were blinded as to whether their dog was receiving sildenafil or a placebo. In a clinical scenario, this information may be considered practical and valuable compared to a more tightly controlled in-hospital protocol. The gold standard for evaluating the LES is measuring pressures via manometry (Kempf et al., 2014; Ullal et al., 2016). This involves passing a catheter down the esophagus and can be a challenging technique to perform in dogs that are awake. For safety reasons, dogs with ME could not have their assessment done while sedated. In addition, monometry is expensive and has limited availability. Videofluoroscopy has been shown to be a safe and effective method of assessing esophageal function in dogs with ME without requiring sedation. For these reasons, VF was chosen for this study.

CHAPTER FIVE: CONCLUSION

Sildenafil may benefit some dogs with megaesophagus by reducing the frequency of regurgitation they experience, thereby improving the prognosis and reducing the morbidity and mortality in dogs affected by this disease. The lack of a significant difference in the change in owner-perceived quality of life or clearance time of food suggests that the positive effects may be influenced by the severity of ME and individual responses. Future studies with a larger number of dogs would be beneficial to continue to assess use of this medication.

Footnotes:

a. Sid's Pharmacy, Pullman, WA 99163

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APPENDIX

Washington State University Owner Consent Form:

Liquid Sildenafil in Canine Megasesophagus Consent Form

Researchers: Susan Mehain, DVM (Small Animal Internal Medicine Resident); Jillian Haines, DVM, MS, DACVIM (Small Animal Internal Medicine)

Purpose of this form:

We would like to include your pet in a research study designed to determine if liquid sildenafil improves clearance of food from the esophagus in dogs with megaesophagus. We are also interested in comparing the period prior to treatment and the period following treatment with sildenafil or a placebo, to determine if there is a noticeable change in clinical signs. This form provides information to help you decide if you want your pet to be in the study or not.

Please read this form carefully. You can ask questions about the purpose of the study, possible risks and benefits, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want your pet to be in the study or not.

Purpose of this study:

The purpose of this study is to assess if the drug sildenafil could work to relax the lower esophageal sphincter in dogs, allowing food to pass more easily from the esophagus into the stomach. This would potentially reduce the frequency of regurgitation, allowing dogs to have better quality of life, reduced risk of aspiration pneumonia, and lower rates of death/euthanasia.

Study enrollment process:

To be enrolled, your dog must have: previously been diagnosed with megaesophagus, classified based on the underlying cause; >1 year of age; and otherwise healthy with no change in clinical signs within the previous 3 months. Enrollment in this study will include: a complete physical examination, a complete blood count, biochemistry panel, urinalysis, chest x-rays, and videofluoroscopy. Evaluation occurs over 3 days total, at two separate visits to the WSU-VTH.

Day 1, your dog will have bloodwork performed, and chest x-rays taken to screen for aspiration pneumonia. They will be given water and food mixed with a contrast agent so we can measure the time it takes for the material to move from the esophagus into the stomach. This is done by fluoroscopy (an x-ray movie), and your dog must sit in a Bailey chair. They will be fed different consistencies while we take fluoroscopic videos every 5 minutes for up to 30 minutes. Your dog will get at least a 2-hour break between sessions, which will each last 30-60 minutes. The following day (**day 2**), you dog will receive either sildenafil or a placebo and have imaging repeated. Following this, your dog will be sent home with either sildenafil or placebo medication, to be given as directed twice daily before meals for 2 weeks. You will be asked to keep a record of regurgitation characteristics for 3 weeks (2 weeks on drug/placebo, 1 week off).

Day 3 of imaging will take place a minimum of 3 weeks later. Your dog will receive the alternative treatment to day 2 (sildenafil or placebo) and imaging will be repeated. Your dog will

again go home with 2 weeks worth of medication (sildenafil or placebo) and you will be asked to record regurgitation characteristics for 3 weeks.

Withdrawal from the study will occur for any dogs that are aggressive, unwilling to eat during imaging, will not tolerate a Bailey chair, or appear distressed during the study.

Potential risks:
Blood collection may be associated with bruising at the collection site. Videofluoroscopy is a type of x-ray, and can emit radiation doses up to 1-3mSv. High doses of radiation may cause cancer in some individuals. Sildenafil appears to be well tolerated by dogs, and will be given at standard doses. Adverse effects are uncommon, but include vomiting and diarrhea. Swallow studies in dogs with megaesophagus carry a risk of aspiration and development of pneumonia; the risk of in-hospital aspiration is considered to be the same as would be expected when feeding at home. If your dog has evidence of aspiration pneumonia during the study period, they will be excluded; you will not receive compensation if this occurs.

Cost of the study:
You will incur no costs associated with the tests or diagnostic procedures performed as part of this study. There will be no compensation for participating in this study. The total cost of services paid for by the study is approximately $600.00.

Additional information:
Enrolling your dog in this study is voluntary and you can withdraw permission and your dog from the study any time. You understand that your dog can be withdrawn from the study if the investigators find it necessary. If your dog is withdrawn for any reason, data already collected may continue to be used for research. Your dog will not be treated differently if you decline to participate in the study. Your decision to participate, not participate, or withdraw your dog from the study will not affect your relationship with WSU or any other treatment your dog receives.

Owner's Statement:
This study has been explained to me. I agree that my dog can take part in this research. I have had a chance to ask questions about the research, with the researcher listed. If I have additional concerns, I can call WSU Institutional Animal Care and Use Committee (IACUC) at (509) 335-7951. This study has been reviewed and approved by the WSU IACUC for using client owned animals for research. I will receive a copy of this consent form. I certify that I am the legal owner or custodian of the dog and have the authority to consent medical treatment for this dog.

Name of owner: _____Signature: _____Date: _____

Names of primary investigators: Susan Mehain, DVM (Internal Medicine Resident); Jillian Haines, DVM, MS, DACVIM (Small Animal Internal Medicine)

Signature: _____Date: _____