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STRESS IN SHELTER DOGS AND THE USE OF FOSTER CARE TO IMPROVE ANIMAL WELFARE

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ABSTRACT

Companion animals are an integral part of families in the United States and improving their well-being has become high priority. Many pets spend time in shelters before finding a permanent home, and their experiences at a shelter can greatly influence their adoptability. To increase adoption rates, it should be the shelter's priority to increase animal welfare by reducing stress. However, with limited resources this can be a difficult task. One affordable option is to implement a foster care system into animal shelters. Unfortunately, there is limited information about the effect of foster care on stress levels in shelter pets. Therefore, this study sought to determine if stress is reduced in shelter dogs, when the animal is entered into foster care.

Saliva was collected from shelter dogs periodically throughout their time at the shelter and their time in the foster home and analyzed for cortisol concentration using an enzyme-linked immunosorbent assay (ELISA). The study also measured stress by quantifying common behavioral and health qualities associated with stress.

Results showed that dogs had higher ($p<0.01$) cortisol concentrations in their first three days at the shelter than they did in their first three days in the foster. This indicates that dogs are more stressed in the shelter than they are in the foster home and thus foster care could be an effective way to reduce stress in shelter pets.

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I would like to thank Centre County PAWS for allowing us to use their dogs in our study. I would also like to thank the foster owners who agreed to participate in the study by collecting saliva from their foster pets. Salivary collection at PAWS would not have been possible without the help of my research assistants Tiffany Bryla and Deanna Trearchis and thanks to Lindy Steinberger for her assistance in running saliva assays to determine cortisol concentration. I would like to thank my statistical consulting team, Kevin Quinlan and Yao Xiong, for helping me with my statistical analysis. I would also like to thank Dr. Nancy Dreschel for her support and encouragement in all stages of this process and Dr. Ott for his assistance and suggestions. Finally I would like to thank my friends and family for supporting me through this process and more. This research project was supported by the Penn State Summer Discovery Grant and the College of Agriculture Summer Research Grant.

Chapter 1

Introduction

Companion animals have become an integral part of families in the United States and improving their well-being has become high priority. Many pets spend time in shelters before finding a permanent home, and often an animal's experience at a shelter can greatly influence their adoptability and the success of the adoption (Mondelli et al., 2004). According to the ASPCA, there are 5 to 7 million companion animals housed in animal shelters each year. There are approximately 5,000 shelters nationwide with little central regulation (ASPCA | Pet Statistics, n.d.). Studies have shown that time spent in shelters can be very stressful and result in elevated cortisol levels, as well as the development of undesirable stress-related behaviors including aggression, fearfulness and timidity (Henessey et al., 1997 ; Mondelli et al., 2004). These behaviors are often the reason why an animal will not be placed in a home or, if the animal is placed, persistence of these behaviors may lead to the return of the animal to the shelter (Mondelli et al., 2004). Animals that are not placed into a home will be humanely euthanized or, in the case of no kill shelters, the animal will remain in the shelter for an indefinite amount of time. To reduce these outcomes, shelters attempt to increase adoption rates by reducing stress and ultimately improving animal welfare (Prescott, 2004). However, with limited resources available to most shelters, reducing stress can be a difficult task.

1.1 Overview of the Stress Response

Stress is the body's way of responding to external stimuli when there is a threat to homeostasis (Charlton, 1992; Moberg, 2000). Changes in the pattern of hormone secretion in response to stress allow the animal to protect itself from the potential threat. For example, if a zebra sees a lion, a stress response is initiated (Sapolsky et al., 2000). The stress response involves three systems, the sympathetic nervous system, the parasympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. These three systems work together and independently to mount the appropriate stress responses (Del Giudice et al., 2011). The sympathetic nervous response, involves a rapid release of catecholamines, epinephrine and norepinephrine. Following this initial response, a slower stress response is initiated by the HPA axis. The hypothalamus secrets corticotropin-releasing hormone which affects the release of hormones in both the hypothalamus and the pituitary gland; it decreases the secretion of unnecessary hormones, such as GnRH, and increases the secretion of hormones essential to the stress response such as adrenocorticotropin hormone (ACTH). Minutes after the detection of a threat, glucocorticoids (GC), such as cortisol, are released from the adrenal glands (Sapolsky et al., 2000). Glucocorticoid secretion is responsible for the physiological changes that occur during the stress response and GC concentration peaks around 20 minutes after a stressful event (Sapolsky et al., 2000; Dickerson & Kemeny, 2004). These changes include diversion of energy to muscles, decreased immune and reproductive function, and increased cognitive function (Sapolsky et al., 2000). All of these changes enable the zebra to maximize its ability to escape the lion.

When a dog experiences a threat to its well-being, it will mount this same response. The closest ancestor to the modern dog (C. lupus familiaris), the wolf (Canis lupus), may experience stress during a hunt, when protecting pups from predators or in pack disputes; however, our domestic dogs do not generally experience these typical stressors. Our domestic dogs do experience the stress of unfamiliar situations. A dog going to a kennel or a dog that is left home alone may experience stress (Henessey et al., 1997). A dog may also mount a stress response during a thunderstorm because it perceives the loud noise and flashing lights as a threat (Dreschel & Granger, 2005). In these situations, dogs often mount a stress response that exceeds the threat to its well-being. A study conducted by Dreschel and Granger (2005) determined that thunderstorm-phobic dogs experienced a 200% increase in salivary cortisol concentrations 20 minutes after termination of a thunderstorm recording. After 40 minutes, the dogs still exhibited salivary cortisol concentrations that were 150% higher than their baseline cortisol levels.

1.2 Stress Experienced in the Shelter Environment

One novel situation that domestic dogs experience is the entrance into a kennel situation, such as an animal shelter. The dog is separated from its human companions and possibly other dog companions and the dog is exposed to unfamiliar surroundings, routines and noises, along with unfamiliar animals. Hennessy et al. (1997), were among the first behavioral scientists to analyze the levels of stress that dogs experience in a shelter situation. They demonstrated that dogs housed in an animal shelter exhibited increased plasma cortisol concentration compared with dogs in a control group that stayed in their familiar homes. Interestingly, the study also noted that cortisol levels decreased with increased periods of time in the shelter and that dogs experienced the most stress in the first three days in their new environment (Hennessy et al., 1997).

To reduce stress in shelter pets and therefore increase their welfare, several studies have been conducted to determine what types of interactions and enrichment activities help relieve stress (Coppola et al., 2006; Menor-Campos et al., 2011). In one study, the effects of human contact on cortisol concentrations in shelter animals were examined. The study design utilized two treatments; positive human contact on day two of arrival at the shelter and reduced human contact. Saliva was collected from the dogs in both groups on days two, three, four and nine and their salivary cortisol concentrations were compared. The study found that dogs that received human contact had overall lower salivary cortisol concentrations than dogs that did not receive human contact, with the biggest difference in concentrations occurring on day three (Coppola et al., 2006). A similar study testing the effects of 25 minutes of human contact on two separate days in the shelter (days seven and nine) also found that the dogs in the treatment group had lower salivary cortisol concentrations than the dogs in the control group. In the same study, the effects of extended exercise sessions on salivary cortisol concentrations were also tested. The exercise sessions which coincided with the human contact sessions also decreased cortisol levels; however, because the two treatments (exercise and human contact) were performed together, it is uncertain which was responsible for the effect (Menor-Campos et al., 2011).

One affordable intervention that has the potential to reduce stress among shelter pets is the implementation of a foster care system into animal shelters. Foster care allows an animal to live with a shelter volunteer until it is adopted into a permanent home. The foster owner becomes the animal's primary caregiver and brings the animal to the shelter only for showing and medical appointments. If, in fact, the shelter situation causes distress in dogs, then removing them from this situation would be an effective means to reduce stress and improve overall animal welfare. Unfortunately, there is not much known about the success of foster care in the shelter system. In fact, no studies reporting differences in cortisol levels in the same dogs living at a shelter and living in a home have been reported. As mentioned earlier, Hennessy et al. (1997), compared cortisol levels between two different groups of dogs, those living at home and those living in a shelter, to see if dogs were more stressed in a shelter (Hennessy et al., 1997). However, it can be misleading to compare cortisol levels between different dogs rather than within the same dog because every dog experiences stress differently. Although one dog may appear to have low levels of cortisol compared to a kennel-mate, that concentration may be high compared to its baseline cortisol concentration. One study examined the reverse situation in which puppies were raised in a home and then transferred to a military base kennel for training (Rooney et al., 2007). In that study, a baseline cortisol concentration was gathered a few weeks before the puppies were moved to their new kennel homes. Once in the training center, urine samples were collected to determine cortisol concentration for the first ten days after arrival. The results of the study demonstrated that cortisol concentration was significantly higher for dogs once they entered the kennel situation compared to their baseline taken from the home (Rooney et al., 2007).

1.3 Salivary Cortisol as a Measure of Stress

Cortisol is a glucocorticoid released in the secondary stress response (Sapolsky et al., 2000). Cortisol concentration has been the standard measure for stress in dogs in many studies (Dreschel & Granger, 2009; Hellhammer et al., 2009; Hennessy et al., 1997). Cortisol can be measured in fecal matter, urine, plasma and saliva. Each medium has its challenges and due to the nature and limitations of this study, salivary cortisol was chosen as the best measure of cortisol. Salivary cortisol shows the dog's current stress levels and, as it is non-invasive, it allows for minimal stress induced by more invasive techniques such as collection of plasma (Lewis, 2006). Collection generally requires minimal restraint, thereby further reducing stress experienced by collection which may skew results. Another advantage to collecting saliva for analysis is in the mode of entry by hormones into this fluid pool. Because most hormones enter the saliva via simple diffusion, the flow rate of saliva should not affect the concentration of specific hormones (Lewis, 2006). Although cortisol is shown to be a good measure of stress, cortisol concentrations are variable depending on the animal. Thus the use of complimentary assays is necessary. Behavioral and health assessments will also be used to assess the stress level of dogs in the shelter and in the foster home.

1.4 Behavior Assessment

Another essential component in assessing stress levels is monitoring animals for behaviors that are correlated with high stress. In a study conducted by Hekman et al. (2012), it was determined that head resting, panting and lip licking were all significantly correlated with cortisol concentrations. Head resting showed a negative correlation while panting and lip licking had positive correlations with cortisol concentration (Hekman et al., 2012). Using this information, and information from an animal behavior video (The Language of Dogs, 2007), a standardized behavioral assessment was developed for this study and used as a check-list in monitoring the animal's behavior.

1.5 Goals and expected outcomes

This study will determine if stress is reduced in shelter dogs when the animal is entered into foster care. It is expected that cortisol levels will be high in the initial transition period to the shelter, with levels decreasing over the period of time spent in the shelter. This trend is predicted due to the expectation that the subject will become adapted to the shelter environment. Once the dog enters the foster system, an initial spike in cortisol levels is expected as the animal settles into its new environment. Again, cortisol is expected to drop over the period of time that the animal is in the foster home. Salivary cortisol concentrations measured in the foster home are expected to be lower than those in the shelter in the same dog, indicating that the foster situation is less stressful for the dog. Due to the high degree of variation between different animals, all measures will be compared within and not between dogs.

This study will also examine correlations between cortisol levels and conditions such as the dog's origins (where it came from, how long it lived there, etc.), pre-existing conditions of the shelter and behavioral traits. The dogs in this study were transferred to the shelter from a variety of situations. Some may have lived in one home for several years and others came from another shelter or where captured as a stray. It is expected that study participants that came from a home to the shelter will experience higher levels of cortisol in the transition period due to the novelty of the situation than dogs that came from another shelter or that were captured as strays. Similarly, dogs that have lived with a family for more than one year are expected to have higher cortisol levels in the transition period than dogs that have not been with a family for very long. The shelter where the study will be conducted has one kennel side that has sound mitigating panels and one non-sound mitigated kennel side, and it is expected that the reduced noise in the sound mitigated side will aid in reducing stress as expressed by lower cortisol concentrations. It is also expected that rejection of food and loose stool will be correlated with higher cortisol concentrations and, as such, could be used as measures of high stress in future studies.

Chapter 2

Methods and Materials

2.1 Study Design

Saliva samples were collected from study participants as described below and analyzed for cortisol concentration. New dogs arrived at the animal shelter on Sundays and collection began on the following Monday morning. Saliva was collected for the first three consecutive days at the shelter, as this is a typical adjustment period for dogs to a new environment (Hennessy et al. 1997). After this critical period, samples were collected once per week for three weeks (6 collections total) or until the dog was adopted or put into foster care (Figure 1). Samples were then taken in the same fashion in the foster home (6 collections total).

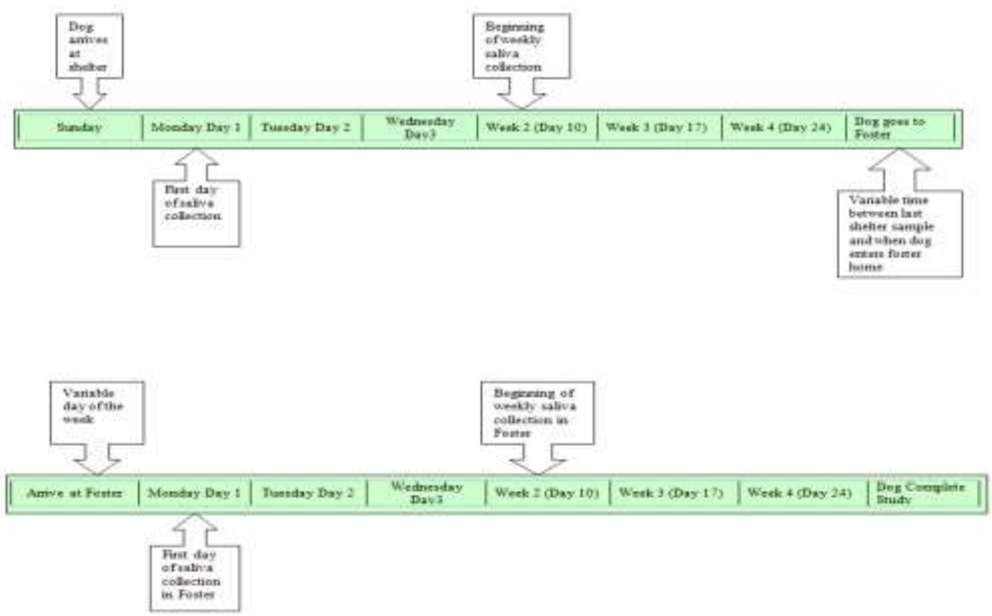

Figure 1 Progression of one study dog from shelter to foster

2.2 Subjects

Subjects for the study were selected from shelter dogs at Centre Country PAWS, a no kill animal shelter. As a no kill shelter, PAWS dogs can live in the shelter for an indefinite period of time, and in extreme cases, dogs have been known to be at the shelter for over two years. However, dogs are generally in the shelter for a few weeks to a few months. Because PAWS has a very successful foster program, it was selected as the shelter of choice for this study. Animals were enrolled in the study between May 13, 2013 and December 2, 2013. New dogs arrived at PAWS on Sunday and salivary collection began the following morning. Enrollment criteria included the following: dogs must be at least 9 kg, 5 months of age or older and in good health. A weight limit was included to aid in saliva collection. Small dogs do not often produce enough saliva for collection in a short time period and extended collection periods will affect the resulting cortisol concentration (Kobelt et al., 2003). Puppies were not included in the study due to developmental processes that may affect cortisol concentrations.

A total of 44 dogs were entered into the study. Of those, 11 dogs provided complete samples (at least 3 samples from the shelter and at least 3 from the foster home). Dogs were omitted from the central study if they did not go to a foster home before being adopted, or if there was insufficient saliva collection in the foster home. Shelter samples from dogs that were omitted from the central study were used in other analyses outside of the central study. The study contained 14 different breeds and breed crosses (see Table 1). There were 23 males and 21 females and the majority (32) of study dogs were altered with six of them unaltered throughout the study period and six dogs were spayed or neutered during the study period. This was noted to evaluate changes in cortisol due to the procedure. Dogs range in age from 0.5 years to 12.7 years and their weight ranged from 9.07 kg to 50.8 kg.

Table 1 Dog breeds included in study

Breed	Number of Dogs
Beagle Cross	7
Lab Cross	6
Beagle	4
Boxer Cross	3
German Shepherd	3
Hound Cross	3
Husky Cross	3
Pit Bull	3
Lab	2
Cocker Spaniel	1
Coonhound	1
English Bull Dog	1
Mastiff	1
Shibainu Mix	1

2.3 Salivary collection

Dogs arrived at the shelter on Sunday evening and were placed in either the sound mitigated or non-sound mitigated kennel (noted for statistical analysis). Saliva was collected from each dog the following Monday morning. All samples were collected between 0800 and 0900 h, prior to the morning meal and after a short walk.

Non-toxic, absorbent polymer children's saliva swabs (Salimetrics, LLC, State College, PA) were used to collect saliva, while the dog was handled or restrained by a research assistant (see Figures 2 and 3). Holding one end of the collection swab, the investigator placed the other end into the dog's mouth for approximately 1-2 minutes, or until the swab felt moist to the touch. The smell of a beef treat was used to stimulate salivation and the dog was rewarded with this treat after collection was complete. Collection was limited to a four minute time frame to avoid effects on stress associated with animal restraint (Kobelt et al., 2003). Any swabs that had blood on them from the dog's mouth were discarded and collection would start over again. If there was only a little bit of blood on the tip of the swab, that end was cut off and the original sample taken before the presence of blood was retained. The samples were immediately frozen and stored at -20º C.

Figure 2 Absorbent polymer saliva swab and storage tube

Once a dog entered foster care, saliva was again collected for three consecutive days followed by weekly collections for three weeks thereafter or until the dog was adopted. For standardization purposes, daily environmental temperatures were recorded to make sure that the kennel temperatures were relatively stable. In the foster home, saliva collection was performed in the same manner as described above; however, collection was done by the foster owner to reduce stress from a stranger in the home. Foster owners received collection swabs and collection tubes along with detailed instructions on how to collect saliva from the dogs. The samples were stored in their home freezers until the collection period was complete, at which time the foster owners brought samples to PAWS on ice and placed them in the freezer to be collected by the investigator. All procedures performed on animals were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC).

Figure 3 Investigator demonstrating sample collection

2.4 Behavioral and Health Assessment

In addition to analyzing salivary cortisol, health assessments were used as another indicator of stress. The shelter has an existing weekly health log that records irregular bowel movements, and eating behavior along with medications and other medical problems that occurred during the week. The information is recorded by the animal shelter volunteers based on daily observations. Another form of assessment used was behavior. A standardized assessment (Appendix A) was developed that includes information regarding the dog's location in the pen, time spent pacing, whining or panting (a fast, dry pant), positioning of the ears and tail, and eating habits and irregular bowl movements. All of these behaviors have been associated with increased stress, but not all of them have been correlated with elevated cortisol concentration (Kalnajs, 2007). When the dog was in the foster home, this assessment was used to monitor frequent stress behaviors in study dogs. Behavior was monitored on a daily basis to determine if stress-related behaviors were noted while in the foster home. Foster owners were asked to complete these general observations of daily behavior. Behavioral comments were grouped together and coded in the datasheet.

2.5 Analysis

Once samples were complete (at least three samples from the shelter and three from the foster home), they were analyzed for cortisol concentration. Samples were thawed and centrifuged at 1500 x g for 15 minutes then pipetted into an enzyme-linked immunosorbent assay (ELISA) kit (Salivary Cortisol kit, Salimetrics, LLC, State College, PA) for analysis of cortisol concentration. Assay range was 0.012 to 3.0 ug/dL. The assays was tested against a broad array of structurally related steroid hormones and shown to exhibit <1% cross-reactivity. Each sample was run in duplicates of 25 µL per well. Some samples did not contain enough saliva for duplicate measurements. The immunoassay plate was coated with antibodies for cortisol. Cortisol in the samples competes with a known and constant amount of horseradish peroxidase-labeled cortisol in the assay well for binding to the cortisol antibodies. The cortisol that was bound to the antibodies was not washed away. Cortisol was measured using a reaction of the horseradish peroxidase with tetrmehtylbenzidine (TMB) which was stopped using sulfuric acid, turning the color of each well from blue to yellow (Chard, 1990; Salimetrics, 2011). The amount of cortisol was determined by measuring absorbance at 450 mn using a Wallac plate reader (Perkin Elmer/Wallac, Waltham, MA), and myassays.com was used to convert the color intensity into cortisol concentration.

All data were analyzed using SPSS predictive analytics software (IBM, Armonk, NY). Descriptive statistics were performed on all subjects that had at least the first sample in the shelter. Due to heterogeneity of error variance, cortisol data were $\log_{10}$ transformed prior to analysis. Untransformed means (arithmetic plus or minus SD) are presented. A linear mixed-effect model was used for analysis fitting the fixed effects of treatment (shelter versus foster home), day (day one, day two, day three) and the interaction between treatment and day with the individual effects of each dog as independent effect (Baayen, et al., 2008). This model was also used to compare cortisol between different situations; such as where the dog came from and background information, such as health, gender and age. A Mann-Whitney U test with original cortisol concentrations was used to compare the cortisol concentrations between dogs that ate and dogs that did not eat and between dogs that had fecal inconsistency and dogs that did not.

Chapter 3

Results

3.1 Subject Characteristics

A total of 39 dogs were included in the statistical analysis. Dogs that did not have samples from the first three days were eliminated from the study, and one dog was omitted due to abnormally high cortisol levels over 49 standard deviations from the sample mean. There was a nearly equal distribution of males and females (53.8% male, 46.2% female). During the first week of collection 66.7% of dogs were already neutered prior to the unset to the study, 28.2% were un-neutered and there was no information on neutering status for two of the study subjects. During the study, six of the ten un-neutered dogs underwent neutering surgery (2 spays, 4 castrations). The weight distribution of the dogs was 9.07 kg to 50.8 kg with an average of 23.4 kg (SD 9.43). Ages ranged from 0.5 years to 12.7 years with a mean of 4.82 years (SD 3.13). Fifteen of the dogs in the study tested positive for Lyme disease upon entrance into the shelter and were administered antibiotics.

Dogs in the study entered the shelter from a variety of backgrounds. The most frequent reason for surrender was that the dog was not good with children or the family was having a baby (n=9). Other common reasons included: cannot take care of (n=6), cannot afford (n=6), too much to handle (n=3), not enough time (n=3), excessive barking (n=3), destruction of property (n=3), moving (n=2) and owner died (n=1). The majority of study subjects were surrendered to the shelter from a home (59%). Fewer dogs came from another shelter before PAWS (30.8%) and even less were found as strays (7.7%).

Of the dogs that were surrendered from a home, 72.7% had lived in that home for more than one year and only 27.3% had lived in that home for one year or less. Of the dogs surrendered from a home, the majority (61.9%) had only lived in that one home as opposed to living in multiple homes (38.1%).

The shelter had two kennel sides and a small dog room, one kennel side had recently installed panels to reduce noise and the other did not. Dogs were randomly assigned to one side or the other when they enter the shelter and there was a fairly even distribution of study dogs between the two kennels. Only one dog was entered into the little dog room (sound mitigated kennel = 43.6%, non-sound mitigated = 51.3%, little dog room =2.6%).

3.2 Salivary Cortisol

Figure 4 shows the cortisol concentrations for study dogs during day one, two and three at the shelter. The mean cortisol concentration for day one at the shelter was 0.818 µg/dL (SD 0.554, n=39), for day two at the shelter the average was 0.633 µg/dL (SD 0.399, n=37) and for day three at the shelter the average was 1.01 µg/dL (SD 1.54, n=37). By the time weekly collections began, many dogs had been adopted or entered into a foster home and thus less data were available. The mean at week two (day 10) was 0.443 µg/dL (SD 0.306, n=27), week three (day 17) was 0.504 µg/dL (SD 0.455, n=25) and at week four (day 24) the average was 0.504 µg/dL (SD 0.374, n=17). There was a tendency for cortisol concentrations to decrease ($p=0.069$) throughout the time at the shelter. The averages for all the shelter samples are presented in Figure 5.

Figure 5 also shows the cortisol concentrations for study dogs during their time in the foster home. The first day in the foster home had an average cortisol concentration of 0.656 µg/dL (SD 0.631, n=10), the second day in the foster home had an average of 0.508 µg/dL (SD 0.592, n=7), the third day in the foster home was 0.396 µg/dL (SD 0.392, n=9). By the time weekly collections began, many dogs had already been adopted and thus less data were available. At week two (day 10), cortisol concentration averaged at 0.369 µg/dL (SD 0.345, n=4), week three (day 17) was 0.201 µg/dL (SD 0.097, n=3) and the fourth week (day 24) was 0.469 µg/dL (SD 0.217, n=2). Cortisol concentrations tended to (p=0.069) decrease over time at the foster home (Figure 5).

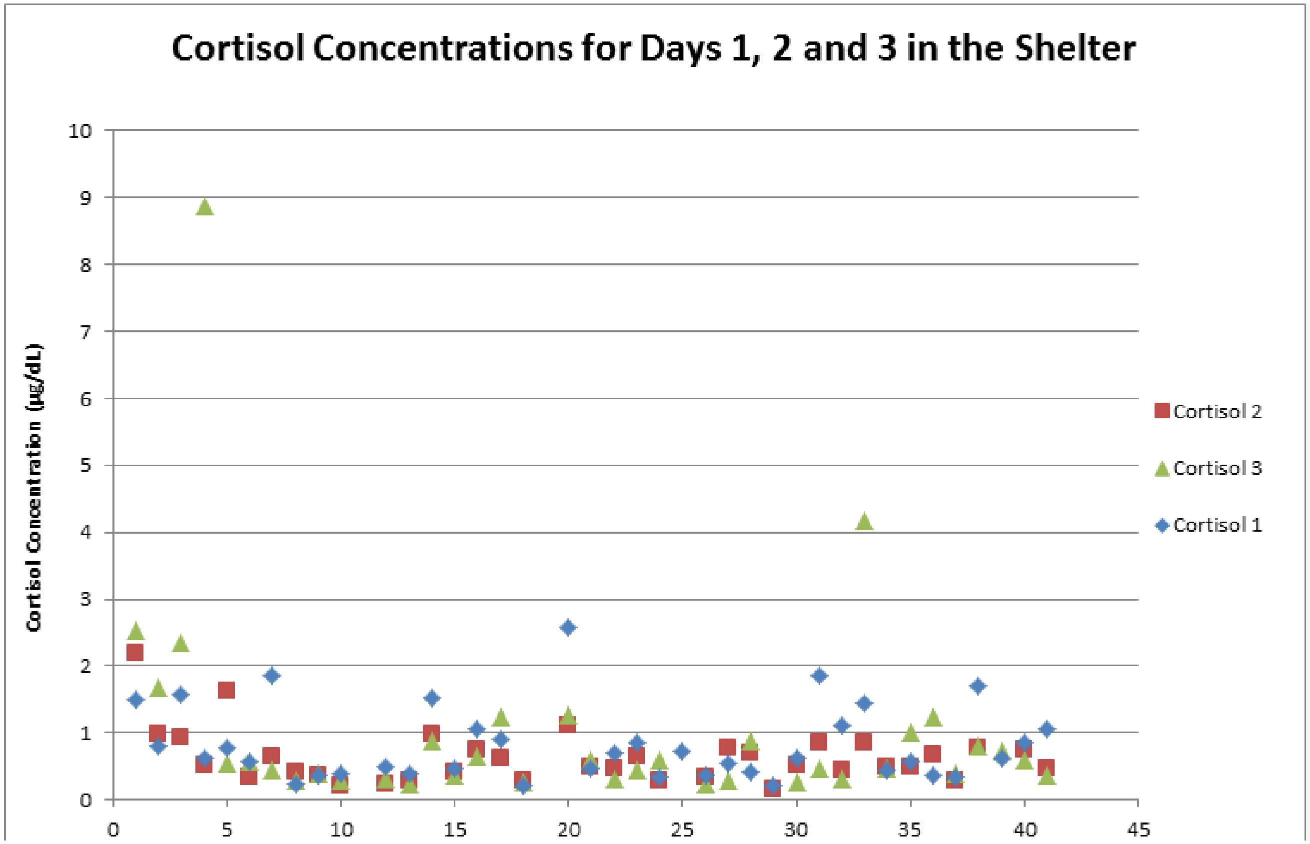

Figure 4 Cortisol concentrations for days 1, 2 and 3 in the shelter

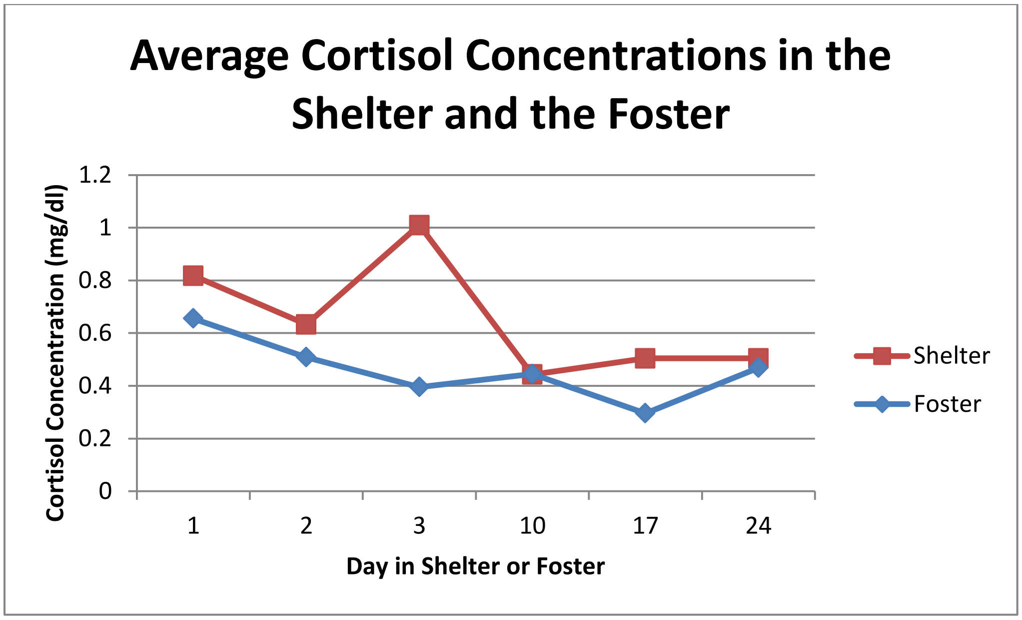

Figure 5 Average cortisol concentrations throughout time in the shelter and time in the foster

Because cortisol concentration exhibited heterogeneous variances, the data were transformed ($\log_{10}$) prior to analysis. Figure 6 shows the normal distribution obtained after transforming cortisol into $\log_{10}$ cortisol and figures 7 and 8 show the distribution of data between different days and treatments (first three days in the shelter and foster, and shelter versus foster treatment).

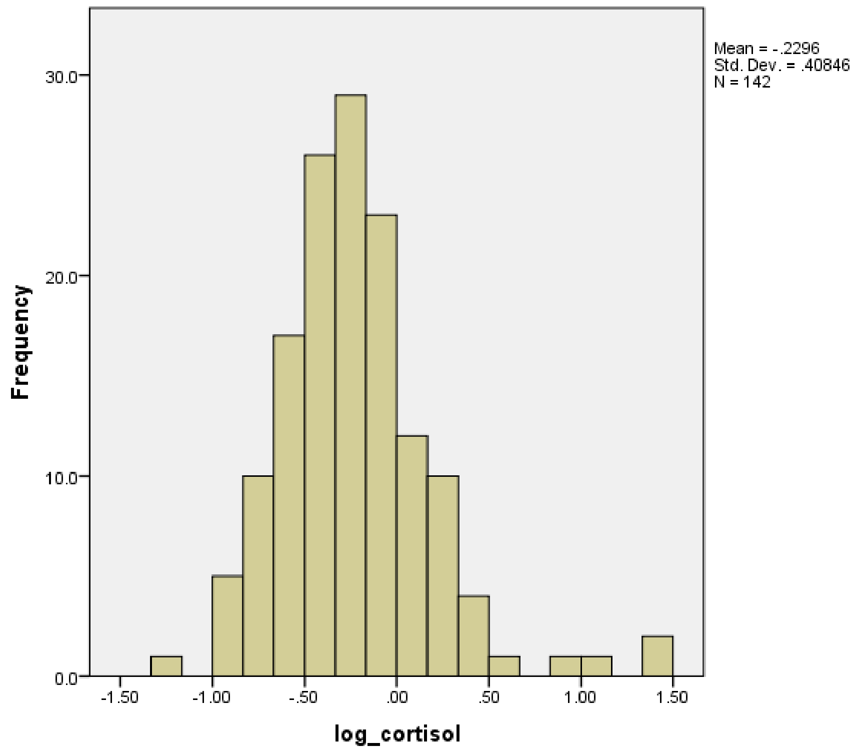

Figure 6 Distribution of log cortisol concentrations

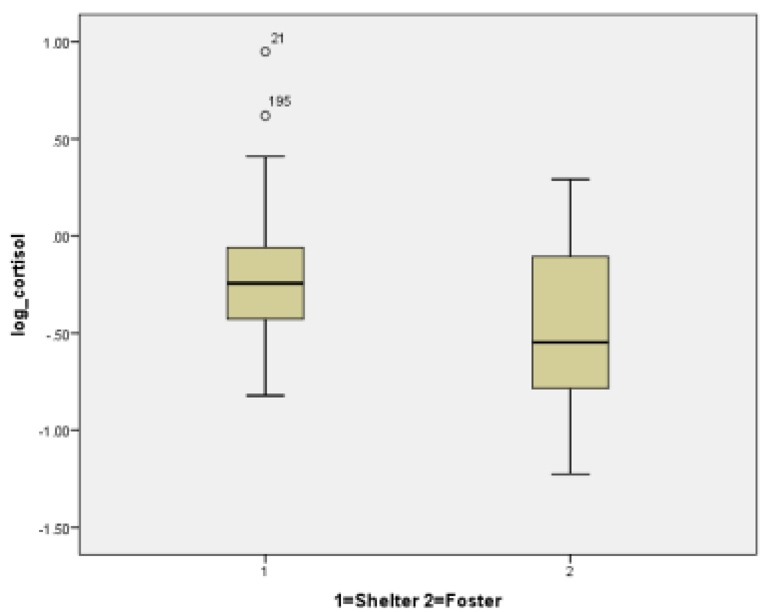

Figure 7 Distribution of log cortisol in the shelter and in the foster

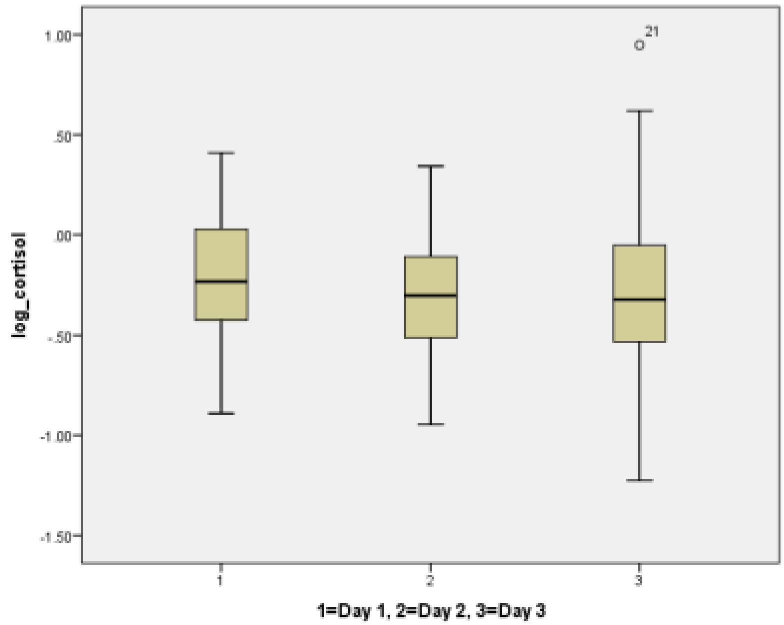

Figure 8 Distribution of log cortisol across day 1, 2 and 3

To compare the data from the shelter and the foster, a linear mixed-effects model was used (Baayen, 2008). Time (day one, two or three) and treatment (shelter or foster) were fixed effects and individual dogs were variable effects. There was no main effect of time (whether the sample was taken on day one, two or three) on cortisol concentration ($p>0.05$). Although overall trends were observed between the different days of treatment, these trends did not reach significance. Salivary cortisol concentrations were higher ($p<0.01$) in shelter dogs than in foster homes. There was no interaction between treatment and time (Table 2). The mean salivary cortisol concentration for the first three days in the shelter was 1.011 µg/dL (SD 0.197) and the mean for the first three days in the foster was 0.469 µg/dL (SD 0.433). Figure 9 shows a graphical representation of mean log_{10} cortisol across different days and in different treatments.

Table 2 Results from mixed effect model

Source	Numerator df	Denominator df	F	P-value
Intercept	1	58.201	66.517	.000
Treatment	1	120.951	22.757	.000
Time	2	97.591	2.756	.069
Treatment * Time	2	97.730	1.035	.359

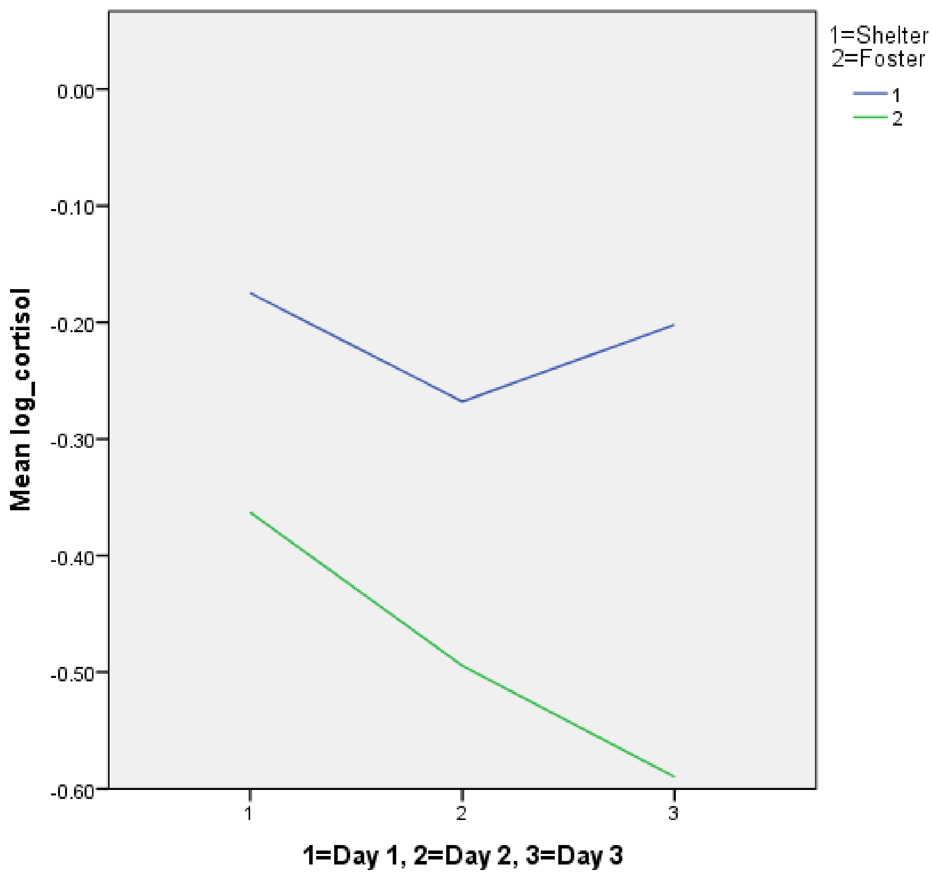

Figure 9 Mean log cortisol over three days of various treatment

For the following analyses, the same linear mixed-effect model was used to test the effects of a particular variable, such as sex, on cortisol concentration. No effects of gender, age, or sound mitigation on salivary cortisol concentrations were detected. There was no effect of origin of the dog (shelter or a home) on salivary cortisol. There was not enough data to determine if stray dogs had different cortisol concentrations than dogs in the other categories. The time spent with one family before coming to the shelter did not affect salivary cortisol. However, if a dog had only lived with one owner before entering the shelter, their salivary cortisol concentration was higher (p=0.019) than dogs that had lived with multiple owners (Figure 10). Dogs that had lived with one owner had a mean cortisol concentration in the shelter of 1.12 µg/dL (SD 0.61) and 0.879 µg/dL (SD 1.03) in the foster. Dogs that had lived with more than one owner had an average shelter cortisol concentration of 0.741 µg/dL (SD 0.828) and an average foster home cortisol concentration of 0.590 µg/dL (SD 0.147). There was no difference in cortisol between dogs that tested positive for Lyme disease.

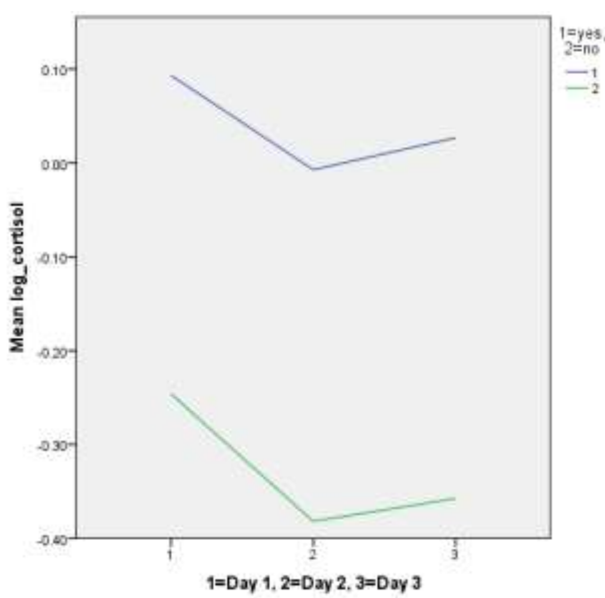

Figure 10 Mean log cortisol across days 1, 2 and 3 between dogs with one owner prior to entering the shelter (1=yes) and dogs with multiple owners prior to entering the shelter (2=no)

The percent changes in cortisol concentration between day one in the shelter and day three were compared using a Mann-Whitney U test against the same variables used above. No difference in the percent change in salivary cortisol was noted between males and females. There was no difference in the percent change in cortisol concentration from day one to day three between dogs living in the sound reduced and the non-sound reduced kennel. There was also no difference between dogs of different origins.

Fecal consistency and eating behavior were compared with cortisol concentrations on day one, two, and three and on week two in the shelter (these collection days showed the greatest prevalence of fecal inconsistency and refusal of food, other collection days did not have sufficient data). Salivary cortisol concentrations in dogs with loose stool were not significantly different than the salivary cortisol concentrations of dogs with normal bowel movements. Dogs that refused food and dogs that ate showed no difference in cortisol concentrations for days one, two and week two; however, on day three, dogs that refused food (M 0.330 µg/dL SD 0.05) had lower ($p=0.006$) cortisol concentrations than dogs who ate their food (M 1.49 µg/dL SD 2.44). No other behavioral analyses were made because there was not sufficient data collected on stress related behaviors.

Chapter 4

Discussion

Salivary cortisol concentrations were higher when dogs were living in the shelter compared to when they were living in the foster home. This indicates that dogs are more stressed when living in a shelter than they are when they are living in a foster home. The shelter averages reported here were similar to the cortisol concentration previously shown in shelter pets (Hennessy et al., 1997). In Hennessy et al. (1997), cortisol concentrations ranged from 0.460 µg/dL to 0.754 µg/dL (depending on the time spent in the shelter). However, in that study, plasma cortisol was measured as opposed to salivary cortisol. There is no literature on cortisol concentration in dogs in foster homes, thus mean cortisol concentration in the foster home cannot be compared with existing research and the difference between shelter and foster cortisol concentrations cannot be compared to existing literature either. However, a similar study which compared cortisol concentrations before introduction into the shelter and while living in the shelter also showed that cortisol concentration was significantly higher when the dog was living in the shelter (Rooney et al., 2007).

Cortisol concentration increased during the transition period (day one to day three) in the shelter, but there was a general downward trend in salivary cortisol concentration over the entire time (to week 4) that did not reach statistical significance. These findings are not consistent with a study conducted by Hennessy et al. (1997) that showed a decrease in cortisol as the number of days in the shelter increased. These differences could be due to the small sample size of this study. With a larger sample size the trends observed may have reached significance. In the foster home, there was a decrease in mean cortisol concentration during the transition period and this trend continued to the end of collection (however, only two dogs had data from day one in the foster to week four). Again these trends were not statistically significant.

Many individual observations were made that can be used to speculate about particular cases. One of the outliers in the study is dog ID 10, whose cortisol concentration at the third day in the foster home was much higher than it was on the first day at the foster home. In reviewing notes, it is clear that this dog was not getting along with their foster owner and showed aggression towards the foster owner’s roommate. On day three, the dog was noted to bite the roommate and shortly after the dog was returned to PAWS. Not all cases can be analyzed in this manner and there is no statistical evidence to support the relationship between the behavior of this dog and its higher cortisol concentration. However, with a larger sample size such individual observations could have significant effects on cortisol concentration.

There were a number of limitations in the collection of saliva for analysis of salivary cortisol concentrations. First, although statistical significance was observed when comparing shelter and foster samples, only 11 samples were considered complete when there were a total of 44 dogs from which saliva was collected. Due to the inability to control when and if shelter dogs were put into a foster home before adoption, the opportunity to obtain many samples was lost. Twenty-two study dogs were adopted into a permanent home before entering the foster care system. Eight dogs had one or more samples omitted because the foster owner was unable collect enough saliva on the swab to obtain 25µL for cortisol analysis. At least two dogs were sent to a foster home, but the foster owner did not agree to participate in the study. One dog's foster data was lost because of an inability to contact the foster owner after sample completion. Samples were never returned to PAWS and attempts to contact the foster owner were unsuccessful. In future studies, these limitations could be reduced by setting up better communication between foster owners and the investigator and by setting up saliva collection workshops for any foster owner participating in the study.

Dog characteristics such as gender and neutering status of the study dogs were analyzed against cortisol concentration to determine if any of these variables showed high cortisol concentrations and could thus be used as potential measures of stress. Using the same linear mixed-effect model, no difference was noted between cortisol concentrations in male versus female dogs. Neutering status was also used as a variable, but there were no significant differences note between the cortisol concentrations of dogs that were neutered and dogs that were un-neutered. These results are supported in research performed by Dreschel and Granger (2005) and Coppola et al. (2006). Both studies showed that the gender of the dog had no effect on cortisol concentration (Dreschel & Granger, 2005; Coppola et al., 2006).

When dogs arrive at PAWS shelter on Sunday, they go through a series of medical tests and evaluations. Lyme disease (*Borrelia burgdorferi),* is a tickborne disease common in Pennsylvania (Jacobs, 2013). Because of its high prevalence in Pennsylvania, all dogs were tested upon arrival at shelter using SNAP-3Dx tests (Idexx, Westbrook, ME). The test gives a qualitative analysis of antibodies in the blood (Littman et al., 2006). About one third of the study dogs tested positive for Lyme disease. Because increased stress causes immune suppression it is possible that dogs with an active Lyme infection could have higher cortisol concentrations (Amadori et al., 2009). However, dogs that tested Lyme positive did not have different cortisol concentrations compared to dogs that were negative for Lyme disease. Furthermore, Doxycycline, which was used to treat dogs testing positive for Lyme disease, did not affect cortisol concentrations.

Prior to the onset of the study, PAWS had installed sound proofing material into one of their kennels. Dogs were randomly assigned by PAWS staff members to either the sound-mitigated kennel or the non-mitigated kennel. One of the stressors involved with living in an animal shelter is the strange and increased noise level (Hennessy et al., 1997). Thus, it was expected that dogs in the sound-mitigated kennel would experience less stress due to decreased noise. However, there was no difference in salivary cortisol concentrations between dogs living in the sound-mitigated and the non-mitigated kennel.

Dogs come to PAWS from a variety of situations including: from a home, from another shelter or rescue center, and found as a stray. It was expected that dogs that entered PAWS from another shelter would have lower cortisol concentration because they would be more accustomed to the kennel environment than dogs that came from a home. However, there was no difference noted in cortisol between these two groups. Among dogs that had entered the shelter from a home, it was expected that dogs that had lived in their homes for more than one year would have higher cortisol concentrations due to separation from their owners. However, no difference was detected in salivary cortisol concentration. Salivary cortisol was higher in dogs that had only ever lived in one home than in dogs that had lived in many homes. This may indicate that dogs that are more accustomed to moving around are less stressed by the shelter situation.

Finally, eating behavior and fecal consistency were examined to determine if dogs that did not eat or had loose stool had higher cortisol concentrations. Shelter dogs have been noted to respond to the stress of an animal shelter by sporadically eating or not eating at all (Miller & Zawistowski, 2004). In several studies conducted in humans, loose stool and conditions such as irritable bowel syndrome are correlated with elevated cortisol concentration (Chang et al. 2009; Walter et al. 2006). Other studies have noted that loose stool in dogs is associated with stress in general (Sokolow et al., 2005). Thus, it was hypothesized that refusal of food and loose stool would be a good indicators of stress in dogs. For this particular analysis, days one, two, three and ten (week two) were examined because these days had the highest incidence of refusal of food and loose stool. Interestingly, no difference in cortisol concentration was detected between dogs that ate and those that did not on days one, two and ten. However, dogs not eating on day three of collection exhibited lower cortisol concentrations. This result is inconsistent with the initial hypothesis of this study. However, there were only seven dogs in the did not eat category on day three at the shelter and one irregular case could have affected the results. No differences were detected between dogs that had loose stool and those that did not. These findings are not supported by the literature that demonstrated a correlation between elevated cortisol and loose stool (in humans) (Chang et al. 2009; Walter et al. 2006). This divergence from the literature could be explained by the small sample size that exhibited loose stool or perhaps loose stool in dogs is not related to cortisol as it is in human subjects. Although none of these variables were associated with cortisol concentrations, this does not mean that they are not indicators of stress. Cortisol is not the only factor involved in stress and these characteristics likely are correlated with other stress indicators. Also, this study had a limited number of dogs and perhaps a larger scale study would produce different results.

Unfortunately, stress related behaviors could not be tested for association with cortisol concentrations. Behavioral assessments were to be collected on a daily basis by PAWS staff members and by the foster owners once the dog entered foster care. Staff members and foster owners were asked to perform the behavioral assessments because they are with the dogs more frequently than the principle investigator and therefore better understood the dog's changes in behavior. However, staff members did not consistently record observations and only a few foster owners returned behavioral assessments with useful information on them. Appendix A shows the behavioral assessment that was developed for this study. Behaviors such as yawning, lip licking, pacing, panting, etc. were all included because of their previously determined correlation to high cortisol concentration or their correlation to other measures of stress (Hekman et al., 2012; Kalnajs, 2007). It was expected that these behaviors and others, including whining, cowering, tail tucking and holding ears back, would be correlated with increased cortisol concentrations and as such could provide good observational measures of stress. In future studies, the principle investigator should be in charge of collecting behavioral data. Or, if daily observation by the principle investigator is not feasible, then it would be beneficial to employ the use of video cameras at the shelter. With the video cameras hooked up to a live feed, the principle investigator would choose a time every day to observe the dogs for a few minutes each.

4.1 Conclusions

Based on reduced overall concentrations of salivary cortisol observed in this study, it is concluded that living in a foster home is less stressful for a dog than living in a shelter. Thus the implementation or increased use of foster care programs in animal shelters could be an effective way to reduce stress in shelter pets. From the results of this study, characteristics such as eating behavior, fecal consistency, dog origins before the shelter and sound mitigation of the kennel were not associated with high cortisol levels. However, the study did find that cortisol was lower in dogs that had lived in more than one home before entering the shelter. Because of the large variation and relatively small samples size, future studies with greater animal numbers are warranted to fully investigate the role of foster care on animal welfare.

In future studies, it would be interesting to see if there is a difference in cortisol concentration in dogs between the foster home and the permanent home. Dogs may become attached to their foster owners and thus the transition to a new home may be very stressful as well. It would also be interesting to look at this study in reverse and see if dogs living in a home experience stress when they enter a kennel while their owners are away on vacation.

Appendix A

Behavioral Analysis

Week of____________ Dog Name and ID ____________________

Eating Habits and Kennel Position

1. Does the dog eat right away when given food or treats?(yes/no)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

2. If not, does the dog eat later?(yes/no)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

3. Where in the kennel does the dog hang out?(front, middle, or back)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

Body Language/other

1. On a scale of 1-5 how often does the dog do the following (with 5 being most of the time and 1 being not at all)

a. Tremble/cower in the kennel

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

b. Pace the kennel or sniff around

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

c. Lay in kennel with head rested

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

d. Yawn or lip lick

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

e. Pant (fast and dry)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

f. Whine or make high pitched noises

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

g. Stand with tail tucked or with tail in an abnormal position for a dog of its breed

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

h. Hold its ears back

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

Overall evaluation

1. On a scale of 1-5 (5 being most stressed) how would you score the dog's stress level?

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

2. Comments on dog's behavior

Sunday	
Monday	
Tuesday	
Wednesday	
Thursday	
Friday	
Saturday	

Initials of Observer ____________

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