

2018

# Physiological and behavioral effects of animal-assisted interventions for therapy dogs in pediatric oncology settings

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## Recommended Citation

McCullough, Amy; Jenkins, Molly; Ruehrdanz, Ashleigh; Gilmer, Mary Jo; Olson, Janice; Pawar, Anjali; Holley, Leslie; Sierra-Rivera, Shirley; Linder, Deborah E.; Pinchette, Danielle; Grossman, Neil J.; Hellman, Cynthia; Guérin, Noémie; and O'Haire, Marguerite, "Physiological and behavioral effects of animal-assisted interventions for therapy dogs in pediatric oncology settings" (2018). *Department of Comparative Pathobiology Faculty Publications*. Paper 37. <https://docs.lib.purdue.edu/cpbpubs/37>

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1 Physiological and behavioral effects of animal-assisted interventions for therapy dogs in pediatric  
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1 **Abstract**

2 **Introduction:** Over the past two decades, animal-assisted interventions (AAIs), defined as the purposeful  
3 incorporation of specially trained animals in services to improve human health, have become increasingly  
4 popular in clinical settings. However, to date, there have been few rigorously-designed studies aimed at  
5 examining the impact of AAIs on therapy animals, despite a notable potential for stress. The current study  
6 measured physiological and behavioral stress indicators in therapy dogs who participated in AAI sessions  
7 in pediatric oncology settings, while also examining the psychosocial effects for patients and their  
8 parents. This manuscript describes the study's canine stress findings. **Methods:** A total of 26 therapy dog-  
9 handler teams were paired with newly diagnosed children with cancer at five children's hospitals in the  
10 United States. These teams provided regular AAI visits to the child and his/her parent(s) for a period of  
11 four months. The teams completed a demographic form, the Canine Behavioral Assessment & Research  
12 Questionnaire (C-BARQ), and a self-report survey to document the types of activities that occurred  
13 during each session. Canine saliva was also collected at five baseline time points and 20 minutes after the  
14 start of study sessions for cortisol analysis, and all study sessions were video recorded to document the  
15 dog's behavior via an ethogram measure. **Results:** Data showed no significant differences in salivary  
16 cortisol levels between baseline ( $0.51\mu\text{g/dL}$ ) and AAI sessions ( $0.44\mu\text{g/dL}$ ),  $p = 0.757$ . Higher salivary  
17 cortisol was significantly associated with a higher number of stress behaviors per session ( $p = 0.039$ ).  
18 There was a significant relationship between stress and affiliative session behaviors ( $p < 0.0001$ ),  
19 indicating that dogs who exhibited more stress behaviors also exhibited more affiliative behaviors. The  
20 dog's most commonly coded session behaviors were oral behaviors, such as lip licking, and tail wagging.  
21 The only C-BARQ factor that was found to have a significant association was stranger-directed fear;  
22 higher scores on this factor were significantly associated with the dog exhibiting fewer affiliative  
23 behaviors in sessions ( $b = 2.12$ ,  $p = 0.042$ ). **Conclusions:** Results show that therapy dogs did not have  
24 significantly increased physiological stress responses, nor did they exhibit significantly more stress-  
25 related behaviors than affiliative-related behaviors, while participating in AAIs in pediatric oncology  
26 settings. The significant relationship between canine cortisol and behavior, thus strengthening the

1 argument for the use of cortisol in canine well-being research. This study discusses the importance of  
2 further investigation to confirm these findings, which may lead to enhanced canine involvement in  
3 hospital settings.

4 **Keywords:** animal-assisted intervention, therapy dog, stress, salivary cortisol, behavior, animal welfare

1 **1. Introduction**

2 Documented benefits of human-animal interactions have increasingly led to the integration of animal-  
3 assisted interventions (AAIs<sup>1</sup>) in services designed to improve human health, and to greater investment  
4 into exploring their effects (McCardle et al., 2011). When included as adjuncts to medical and mental  
5 health treatment, interactions with therapy animals have been shown to distract from or alleviate anxiety;  
6 decrease pain perception; motivate therapeutic participation; normalize clinical environments; and  
7 provide social support (Braun et al., 2009; Fine, 2015; Nimer & Lundahl, 2007; Wu et al., 2002). Dogs,  
8 because of their relative trainability, predictable behavior, and availability are commonly utilized as  
9 therapy animals in a variety of AAI applications and therapeutic settings (Glenk, 2017).

10 Through nearly 35,000 years of domestication, dogs have become well-attuned to nuances in human  
11 emotions, behaviors, and attentional states (Albuquerque et al., 2016; Reid, 2009; Wang et al., 2016).  
12 Studies (Handlin et al., 2011; Odendaal & Meintjes, 2003) show that people who interact with dogs  
13 experience elevated levels of the attachment hormone oxytocin, with recent research (Nagasawa et al.,  
14 2015) demonstrating increased oxytocin for both humans and their pet dogs through mutual gazing. While  
15 heightened sensitivity to the human condition is likely advantageous for connecting with AAI recipients,  
16 it may also pose salient welfare risks to therapy dogs. For example, dogs have exhibited similar  
17 physiological stress responses as humans after listening to infant crying (Yong & Ruffman, 2014).  
18 Because such outward displays of human distress often occur in therapeutic settings, therapy dogs may be  
19 vulnerable to stress during AAI participation (Glenk, 2017). Additionally, as registered therapy dogs are  
20 trained to calmly tolerate interactions that they might otherwise find taxing (e.g., rushed head-petting by  
21 strangers), handlers may find it difficult to notice distress in their dogs who may already be inclined to  
22 please (Glenk, 2017).

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<sup>1</sup> AAI is to be defined as the purposeful incorporation of specially trained and qualified animals, most commonly dogs, in services to improve human health (Nimer & Lundahl, 2007; International Association of Human-Animal Interaction Organizations or IAHAIO, 2014).

1 To date, research regarding the experience of therapy dogs in AAIs has been informative, but remains  
2 limited. Most measures of therapy dog welfare rely on physiological (cortisol), behavioral (stress-related)  
3 and/or observational (handler perspective) indicators, as it is problematic to assess canine stress by any  
4 one indicator alone. Without behavioral context, it is difficult to conclude whether elevated cortisol  
5 indicates distress or eustress (Edgar et al., 2012).

6 While current research suggests minimal welfare concerns for therapy dogs, results have been mixed and  
7 are difficult to generalize given a lack of practice fidelity. Likewise, organizations that register therapy  
8 dogs often differ in their health and safety policies, including inconsistent vetting and training procedures  
9 meant to limit canine stress (Linder et al., in press). Recent research (Haubehofer and Kirchengast, 2006,  
10 2007) found that therapy dogs experience higher levels of physiological stress on working days when  
11 compared to days at home. Salivary cortisol was also higher for dogs participating in shorter and more  
12 frequent AAI sessions, possibly due to heightened intensity or fewer respite opportunities (Glenk, 2017;  
13 Haubehofer & Kirchengast, 2006, 2007). King et al. (2011) reported indicators of canine physiological  
14 and behavioral stress after 1-2 hours of AAI participation in hospital settings, including stress-related  
15 behaviors, increased salivary cortisol, and cortisol-stress behavior correlation. Additionally, a higher  
16 prevalence of behavioral stress has been observed in therapy dogs during interactions with children  
17 younger than 12 years, whose relatively erratic behavior may cause dogs discomfort (Marinelli et al.,  
18 2009).

19 Environmental factors (e.g., strange stimuli/people) may also increase therapy dog stress. Recently,  
20 researchers found that novel settings significantly increased salivary cortisol in therapy dogs when  
21 compared to familiar locations, underlining the importance of allowing dogs to become accustomed to  
22 new AAI environments (Ng et al., 2014). Likewise, handlers have reported such factors as high  
23 temperatures and confined spaces as stressful for dogs (Marinelli et al., 2009).

24 In contrast, other studies have found scant evidence indicative of canine distress during AAIs (Barstad,  
25 2014; Palestini et al., 2017; Piva et al., 2008), including no differences in physiological stress between  
26 working and non-working days (Glenk et al., 2013, 2014; Ng et al., 2014). In research examining cortisol

1 and behavior, Glenk et al. (2014) found that therapy dog behavior did not significantly change over time,  
2 and that salivary cortisol decreased in the final two sessions with adults undergoing substance abuse  
3 treatment.

4 Simply put, AAI practice must be mutually beneficial to be considered ethical, with the needs of therapy  
5 animals prioritized as highly or more so than those of human recipients (Serpell et al., 2010). Given the  
6 potential for canine stress during AAIs, as well as the current lack of agreement regarding if and how  
7 AAIs affect animal welfare, additional research is essential to understand how to best support therapy  
8 dogs as their involvement in human health services broadens. To that end, this study measured the  
9 physiological and behavioral effects of regular AAI sessions for registered therapy dogs in five U.S.  
10 pediatric oncology settings. This research also measured the effects of sessions on patients and their  
11 parents, with these findings reported elsewhere (McCullough et al., in press). In regard to canine  
12 outcomes, we hypothesized that therapy dogs would exhibit minimal indicators of physiological and  
13 behavioral distress over their study participation.

## 14 **2. Methods**

15 All protocols and surveys were approved by the Institutional Animal Care and Use Committee and the  
16 Institutional Review Board at American Humane, as well as at each participating hospital site. The  
17 following five hospitals, located in the United States, participated in this research study: Monroe Carell Jr.  
18 Children's Hospital at Vanderbilt in Nashville, Tennessee; Randall Children's Hospital in Portland,  
19 Oregon; University of California Davis Children's Hospital in Sacramento, California; St. Joseph's  
20 Children's Hospital in Tampa, Florida; and Children's Medical Center at UMass Memorial Health Care,  
21 in partnership with Cummings School of Veterinary Medicine at Tufts University, in Worcester,  
22 Massachusetts. Modifications to the protocol were made at two different time points, which impacted the  
23 human participants' enrollment and retention rates (e.g., broader inclusion criteria for children and a  
24 monetary incentive for control group patients). These changes were not related to any of the canine  
25 protocols.

### 26 *2.1 Participants*



1 Eligible therapy dog-handler teams were identified by the hospital sites' volunteer services coordinator,  
2 study coordinator, and/or by other handlers through a referral process. Each participating team consisted  
3 of a therapy dog and his/her handler, who were registered by a national organization that requires rigorous  
4 training and evaluation policies. These teams volunteered to visit children, ages 3-17 years, who had been  
5 recently diagnosed with cancer, enrolled in the study at one of the study sites, and randomly assigned to  
6 the intervention group to receive regular (e.g., weekly) visits from a therapy dog-handler team for a  
7 period of four months. Prior to participation, all handlers were required to complete the hospital volunteer  
8 services training and credentialing process, and to adhere to the rigorous health, safety, and privacy  
9 guidelines and policies in place at their respective hospital site. To support safe interactions, all therapy  
10 dog handlers had completed extensive AAI training, and all therapy dogs had passed a behavioral and  
11 health evaluation, prior to enrollment. Dogs were required to be a minimum of one year old and have at  
12 least six months of AAI experience with the handler prior to enrolling in the study.

13 Twenty-six teams participated in the study. Prior to consenting, each team attended a study-specific  
14 training at their local hospital site. At this meeting, a detailed description of the study protocol, including  
15 how to collect their dog's saliva and how to complete all other applicable instruments, was presented.  
16 Handlers had the opportunity at this training to practice and demonstrate their ability to properly obtain a  
17 saliva sample from their dog. All handlers received printed training materials to keep for their reference,  
18 as well as online links to videos demonstrating proper saliva collection techniques.

19 Sample size was determined based on a power analysis for the human measures in the study, which  
20 yielded a sample size of 100 patients to be enrolled. Approximately half of this group (n = 60) was  
21 randomly assigned to receive AAI sessions with therapy dogs. To facilitate these sessions for 60 child  
22 participants over a period of 33 months, 26 dogs participated in the canine assessment portion of the  
23 study.

## 24 *2.2 Animal-Assisted Intervention*

25 The human participants who were randomized to the control group received standard care at their hospital  
26 site, meaning each family received services from the oncology team. Human participants randomized to

1 the intervention group received their standard care offered at the hospital plus regular visits from a  
2 participating therapy dog-handler team. Children were enrolled for a four-month period near the onset of  
3 their cancer treatment. Interactions with the therapy dog occurred approximately once per week, and  
4 coincided with the child's treatment schedule and clinic visits. Depending on existing hospital policies  
5 and procedures, children transferred to inpatient care could continue their visits with the therapy dog,  
6 while others could visit in the outpatient oncology clinic only. Children were matched, by the site  
7 coordinator, to a specific therapy dog-handler team who became their primary team for the study; back-up  
8 teams were used on an as-needed basis. Children were matched to their therapy dog team based on the  
9 child's treatment schedule and the team's volunteer availability.

10 All therapy dog sessions were prescribed to last approximately 20 minutes, with session length ultimately  
11 determined by the patient, parent, handler, site coordinator, or another medical staff member. AAI  
12 sessions were nonprescriptive in nature, allowing for flexibility in activities and physical intensity based  
13 upon the child's health status and the level of therapy dog and child engagement. However, all handlers  
14 were given a list of commonly used activities to choose from as a reference guide (e.g., pet the dog,  
15 brushed the dog, practiced dog's cues, etc.). These sessions took place in semi-private to private areas of  
16 the pediatric oncology clinic, depending upon the health status of the child and room availability.

### 17 *2.3 Measures*

18 A variety of instruments were used to assess indicators of canine stress and well-being over the course of  
19 the study.

#### 20 *2.3.1 Demographic Form*

21 Demographic information was obtained from participating handlers using a self-report questionnaire. In  
22 addition to factors such as age, race, gender, and canine breed, handlers reported information about the  
23 length of time they had been volunteering with their therapy dog, with what populations, and in what  
24 types of settings.

#### 25 *2.3.2 Canine Behavioral Assessment & Research Questionnaire (C-BARQ)*

1 After consenting to participate, therapy dog handlers completed the C-BARQ for their dog who would be  
2 participating in the study. This standardized 100-question instrument is completed online and asks dog  
3 owners to indicate how their dog typically responds to common events and stimuli in their environment  
4 (Serpell, 2017). The C-BARQ is comprised of 14 behavioral factors: trainability, stranger-directed  
5 aggression, owner-directed aggression, dog rivalry, stranger-directed fear, non-social fear, dog-directed  
6 aggression, dog-directed fear, touch sensitivity, separation-related behavior, excitability,  
7 attachment/attention-seeking, chasing, and energy. This instrument was utilized as a descriptor for the  
8 therapy dog population's temperament and behavior.

### 9 *2.3.3 Salivary Cortisol*

10 Salivary cortisol is a known bio-marker for stress and arousal in humans and animals (Hekman et al.,  
11 2012; Kirschbaum & Hellhammer, 1994). Dog's salivary cortisol levels lag plasma levels by 20 minutes,  
12 indicating that salivary cortisol peaks roughly 20 minutes after a stressful event, which is then maintained  
13 for approximately 0.5 hours before declining (Hennessy et al., 1998; Vincent & Michell, 1996). In this  
14 study, therapy dog-handler teams collected their dog's saliva at five different time points at the beginning  
15 of the study to establish a baseline cortisol measurement. These five time points were as follows: 1. Upon  
16 their dog waking in the morning, prior to breakfast; 2. Mid-day/noon; 3. Evening, just prior to the dog's  
17 typical bedtime; 4. Approximately 20 minutes after the presence of an AAI-specific "trigger" – such as  
18 the introduction of his/her therapy vest/bandanna or visit bag; and 5. Approximately 20 minutes after  
19 arriving at the participating hospital/clinic site.

20 Given the known salivary cortisol lag and peak times, as well as our flexible approach to session timing  
21 described above, the study protocol specified that therapy dog handlers collect their canine's saliva  
22 sample immediately following a session, except for in cases where sessions lasted fewer than 20 minutes.  
23 In instances where sessions lasted less than 20 minutes, handlers were asked to wait in a quiet room for a  
24 short period to allow for the saliva they collected to accurately represent their dog's response to the  
25 session (e.g., if a session lasted for five minutes, the handler waited in a quiet room for a period of 15  
26 minutes and then began the saliva collection process).

1 Saliva was collected by the therapy dog handler using recommended collection procedures (Dreschel &  
2 Granger, 2009). To collect saliva, the handler sat/kneeled on the floor or a chair, and placed an absorbent  
3 125mm long Salimetrics (State College, PA, USA) SalivaBio Children’s Swab into the dog’s mouth. The  
4 handler would rub the swab around the dog’s cheek and gum area for approximately 90-300 seconds,  
5 until the swab felt saturated with saliva. Saturated swabs were then placed into a saliva collection tube  
6 pre-labeled with the Handler ID, Child ID (for post-session samples), and sample number. Handlers then  
7 hand-wrote the date and time of collection, and gave the saliva collection tube to the study coordinator to  
8 store and ship for analysis. All tubes were stored at -20° C in a medical grade freezer at each hospital to  
9 ensure sample viability. Approximately once every three months, a set of samples were shipped to  
10 Salimetrics, in a Styrofoam cooler packed with dry ice, for testing. Each sample underwent  
11 Salimetrics Cortisol Enzyme Immunoassay Kit, which is a duplicate assay. The results of the assays were  
12 averaged to create one value for the study analyses, unless the saturation amount would not allow for  
13 duplicate, and were calculated as µg/dL.

#### 14 *2.3.4 Therapy Dog Handler and Study Coordinator Self-Reports*

15 After each AAI session, handlers completed a self-report form to indicate the activities that occurred.  
16 They were provided with a list of common AAI activities to choose from, but could also indicate if any  
17 other activities took place that were not pre-defined. This list of session activities was tracked for both the  
18 child and parent using zero-one sampling (i.e., activity occurred or did not occur). Handlers also used this  
19 form to describe any environmental factors related to the session and their dogs’ behavior (e.g., “room  
20 was very warm which affected dog's attention and behavior”, “[dog] intermittently distracted by noise in  
21 hallway”, etc.). The site coordinator filled in who was present and participated in the activities, and  
22 indicated any observations of the child’s or dog’s behavior that they felt was pertinent (e.g., “had to meet  
23 with dog in hallway today”, “child quite nauseated today”, etc.).

#### 24 *2.3.5 Canine Behavior Ethogram*

25 Each AAI session was recorded by two video cameras (Samsung F90 HD Camcorder with 2.7" LCD  
26 Screen) to ensure that the dog’s behavior was captured during the interaction. These recordings were then

1 coded using a non-standardized behavior ethogram developed by the study's researchers and informed by  
2 animal behaviorists and veterinarians. This ethogram included 26 behaviors divided into three categories:  
3 **affiliative indicators** (leaning or resting body or head against a person or object; licking a person;  
4 pawing/paw lifting; play stance/bow; pushing snout; raising ears; rolling over; tail wagging; walking  
5 towards a person), **moderate stress indicators** (body shaking or "shaking off"; escape; looking at/to  
6 handler; looking away; oral behaviors, such as lip licking, extending the tongue, or smacking the lips;  
7 panting - excessive or prolonged; restlessness; self-directed behaviors; yawning), and **high-stress**  
8 **indicators** (baring teeth; barking, yelping, yipping, whining, or whimpering; biting or attempting to bite;  
9 crouching; drooling--excessive or in copious amounts; ears plastered/pinned back; growling; stare gaze).  
10 All behaviors exhibited by the therapy dogs were tallied for their frequency. The total frequency of stress-  
11 related behaviors were summed to create one score (17 items), as were the affiliative-related behaviors (9  
12 items).  
13 Ethogram coders underwent several rounds of training on how to use the instrument, including viewing  
14 video clips of the potential behaviors that were to be coded. Inter-rater reliability coefficients were  
15 calculated at various time points during the training process, and additional training sessions were  
16 conducted until a satisfactory level of inter-rater agreement and coder confidence was established. One  
17 individual served as the primary coder, with two additional coders providing supplementary coding  
18 support.

## 19 *2.4 Settings*

20 Data collection for this study occurred in the handler's place of residence and at the participating hospital  
21 sites.

### 22 *2.4.1 Home Setting*

23 Four out of the five baseline cortisol data points (morning, mid-day, evening, trigger) were obtained at the  
24 therapy dog team's place of primary residence. The morning collection occurred upon the dog waking,  
25 and typically occurred between 05:19 and 10:30 a.m. (M = 07:47 a.m., SD = 01:13 hours:minutes). The  
26 mid-day collection occurred between 07:00 a.m. and 09:15 p.m. (M = 12:40 p.m., SD = 2:41

1 hours:minutes). The evening collection occurred between 10:40 a.m. and 11:00 p.m. (M = 7:57 p.m., SD  
2 = 3:16 hours:minutes). The trigger collection occurred approximately 20 minutes after giving the dog a  
3 signal that they would be going to work (e.g., therapy dog vest/bandanna). The trigger sample was  
4 collected between 07:05 a.m. and 11:00 p.m. (M = 11:11 a.m., SD = 3:12 hours:minutes).

#### 5 *2.4.2 Hospital Setting*

6 The final baseline saliva sample was collected 20 minutes after arriving at the hospital where the team  
7 participated in study sessions. This collection occurred between 08:15 a.m. and 4:45 p.m. (M = 12:16  
8 p.m., SD = 2:08 hours:minutes). The goal of collecting baseline samples at the dog's home and at the  
9 hospital was to obtain a measure of the dog's typical salivary cortisol levels on non-working days  
10 (without any interactions with unfamiliar individuals) to then compare to cortisol collected after each AAI  
11 session.

12 To allow for the therapy dog to have minimal distractions during the collection process, post-session  
13 saliva samples were collected at the hospital site in a quiet, semi-private to private area designated by the  
14 site coordinator and handler. This area was generally separate from where the AAI sessions took place.

#### 15 *2.5 Statistical Analyses*

16 Analyses were conducted to address two key goals: 1). to compare differences in salivary cortisol  
17 between control/baseline and AAI conditions and 2). to examine factors affecting salivary cortisol and  
18 behavior during AAI sessions. Statistical analyses were conducted using mixed modeling procedures  
19 (Raudenbush & Bryk, 2002). This hierarchical analysis technique accounted for the nested data design,  
20 with repeated measurements nested within individual dogs nested within hospital sites. Random factors in  
21 all models included dog and hospital site.

22 Salivary cortisol data were positively skewed and not normally distributed. Therefore, raw values were  
23 log-transformed for further analyses using linear mixed models. Means and graphs present raw values for  
24 ease of interpretation. All models for salivary cortisol control for time of day as a fixed factor. To  
25 compare the effect of study conditions on salivary cortisol, we conducted a linear mixed model with the  
26 fixed effect of condition (morning, mid-day, evening, trigger, hospital, AAI session). To examine factors

1 affecting salivary cortisol, we conducted linear mixed models with additional fixed factors related to  
2 demographics (age, gender, experience, breed), C-BARQ factor scores, or AAI session characteristics  
3 (duration, number of people present and participating, reported activities).  
4 Ethogram behavior data were analyzed using generalized linear mixed models appropriate for count data  
5 with a Poisson distribution sampling method and a log-link function. All ethogram behavior models  
6 control for the length of the video as a fixed factor. To examine factors affecting behavior, we conducted  
7 linear mixed models for stress and affiliative behaviors with fixed factors related to demographics (age,  
8 gender, experience, breed), C-BARQ factor scores, or AAI session characteristics (number of people,  
9 reported activities). We also included the fixed factor of cortisol to examine the relationship between  
10 cortisol and behavior.  
11 Analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 24.0. All  
12 significance tests were two-tailed with a significance level of  $\alpha < 0.05$ .

### 13 **3. Results**

14 The final dataset for analysis included 604 data points, or records, across 26 dogs. At the dog level ( $n =$   
15 26), data included demographic characteristics ( $n = 24$  dogs) and C-BARQ surveys ( $n = 26$  dogs). At the  
16 repeated measures record level ( $n = 604$ ), data included AAI Therapy Dog Handler and Study  
17 Coordinator Self-Reports ( $n = 445$ ), ethogram behavioral data ( $n = 405$ ), and valid salivary cortisol  
18 samples ( $n = 411$ ).

19 The random factors in the mixed models accounted for variance across dogs and hospital sites. The  
20 random factor of hospital site was not significant in any model ( $ICC \leq 0.423$ ,  $p \geq 0.209$ ), indicating that  
21 there were no significant differences in outcomes across hospitals. The random factor of dog was  
22 significant in all models ( $ICC \geq 0.030$ ,  $p \leq 0.046$ ), indicating that there were significant individual  
23 differences across dogs that were accounted for in the analyses.

#### 24 *3.1 Participants*

25 The sample of therapy dog-handler teams with demographic data ( $n = 24$ ; two handlers declined to  
26 provide this information) included dogs aged two to 13 years old, who were primarily female (58%) with

1 0.5 to 9.17 years of experience as a visiting therapy dog. A wide range of dog breeds of varying sizes  
2 were represented, including Miniature Poodles, Newfoundlands, a Border Collie mix, a Dachshund, and a  
3 Wheaton Terrier, while the most common breeds were Golden Retrievers (17%), Labrador Retrievers  
4 (13%), or mixed breeds that included part Golden or Labrador Retriever (16%). Therapy dog handlers  
5 identified as predominantly female (92%), 46 years or older (83%), and Caucasian/White (96%). Therapy  
6 dog-handler teams were largely registered through Pet Partners (75%). Dog handlers had between 0.5  
7 and 10.42 years of experience as AAI handlers, with most of these teams (67%) having previous  
8 experience in hospital settings.

### 9 *3.2 Session Characteristics: Therapy Dog Handler and Study Coordinator Self-Reports*

10 The study included 445 AAI sessions recorded by Therapy Dog Handler and Site Coordinator Self-  
11 Reports. Sessions lasted between five and 180 minutes, with an average of 23.95 minutes per session  
12 (Median = 17; Mode = 15; SD = 21.13). The categories of individuals at each session were noted and the  
13 number of people who were present and participated at each session (excluding the handler) ranged from  
14 one to eight. On average, 3.11 people were present and 2.27 people participated in session activities with  
15 the therapy dog. The most common people *present* included the child (n = 445 sessions, 100%), mother (n  
16 = 377, 85%), father (n = 151, 34%), and nurse (n = 110, 25%). The most common people who  
17 *participated* in session activities included the child (n = 417, 94%), mother (n = 306, 69%), and father (n  
18 = 113, 25%).

19 The most common activities engaged in by the child included petting the dog (n = 409, 92%), talking to  
20 the dog (n = 307, 69%), viewing the dog's photos (n = 144, 32%), and brushing the dog (n = 106, 24%).  
21 The most common activities engaged in by the parent included petting the dog (n = 244, 55%), talking to  
22 the dog (n = 212, 48%), taking a photo of the dog and/or child with the dog (n = 69, 16%), and viewing  
23 the dog's photos (n = 64, 14%). Across 445 recorded sessions, children participated in 1,446 activities,  
24 while parents participated in 801 activities. See Table 1 for a complete list of session activity frequencies.

### 25 *3.3 Canine Behavior Ethogram*



1 AAI sessions were video recorded to code canine behavior via a pre-defined ethogram. Inter-rater  
2 reliability of ethogram behavior data was calculated using a two-way mixed effect intra-class correlation  
3 coefficient. Reliability between the primary and secondary coder was good ( $ICC = 0.782, p < 0.0001$ ). An  
4 average of 9.69 ( $SD = 10.47$ ) stress behaviors and 9.14 ( $SD = 9.33$ ) affiliative behaviors were recorded in  
5 each session. There were no significant differences between the frequency of displayed stress versus  
6 affiliative behaviors,  $t(404) = 1.19, p = 0.236$ . The most common canine behaviors included oral  
7 behaviors, such as lip licking, and tail wagging. There were no high-stress indicator behaviors coded for  
8 any dog across all study sessions. Descriptive statistics for ethogram behaviors are presented in Table 2.  
9 There was a significant association between stress and affiliative behaviors,  $\beta = 0.21, t(402) = 9.39, p <$   
10  $0.0001$ . Dogs who displayed more stress behaviors in a given session also demonstrated more affiliative  
11 behaviors during that session.

### 12 *3.4 Salivary Cortisol*

13 There were no significant differences in salivary cortisol levels between baseline and AAI sessions ( $p$   
14  $range = 0.348 - 0.751$ ; Table 3). Salivary cortisol levels were relatively consistent across all conditions  
15 (Figure 1), including morning ( $n = 24, M = 0.59, SD = 1.40$ ), mid-day ( $n = 23, M = 0.70, SD = 1.62$ ),  
16 evening ( $n = 22, M = 0.40, SD = 0.44$ ), trigger ( $n = 25, M = 0.32, SD = 0.25$ ), hospital ( $n = 22, M = 0.44,$   
17  $SD = 0.60$ ), and AAI sessions ( $n = 299, M = 0.44, SD = 0.82$ ).

18 Canine cortisol levels did not increase over time of involvement in the study, and remained consistent  
19 with their baseline levels.

### 20 *3.5 Relationship Between Demographics, Salivary Cortisol, and Behavior*

21 The relationship between demographic characteristics and canine outcomes are presented in Table 4.  
22 Therapy dog gender was related to salivary cortisol ( $p = 0.002$ ), with female dogs exhibiting significantly  
23 lower salivary cortisol than males. Animal age was related to salivary cortisol ( $p = 0.004$ ), stress  
24 behaviors ( $p = <0.0001$ ), and affiliative behaviors ( $p = 0.010$ ). Older dogs showed lower salivary cortisol  
25 and displayed more behaviors during AAI sessions, including both stress and affiliative behaviors. There

1 were no effects of handler years of experience on salivary cortisol or behavioral outcomes (p range =  
2 0.197 - 0.421).

3 There was no relationship between most C-BARQ factors and canine outcomes (p range = 0.129 - 0.882).  
4 The only factor with a significant effect was stranger-directed fear. Higher stranger-directed fear, as rated  
5 by handlers, was associated with 2.12 fewer affiliative behaviors on average per session (p = 0.042).

6 *3.6 Relationship between session characteristics, salivary cortisol, and behavior*  
7

8 The relationship between session characteristics and canine outcomes is presented in Table 5. Duration of  
9 sessions was not significantly related to salivary cortisol (p = 0.481) or stress behaviors (p = 0.092).  
10 Session length was significantly associated with affiliative behaviors (p < 0.0001), but the effect was  
11 relatively small; therapy dogs displayed 0.01 more affiliative behaviors for each minute longer a session  
12 lasted. There were no significant effects of the number of people present (p ≥ 0.165) or participating (p ≥  
13 0.134) on salivary cortisol or behavior. There were also no effects of the type of session activities on  
14 salivary cortisol (p ≥ 0.105).

15 There were significant relationships between session activity type and behavior (Figure 2). Dogs  
16 displayed significantly *fewer affiliative behaviors per session* when the following activities took place:  
17 participant got water for the dog; child played a game on the dog's vest; child drew a picture of the dog;  
18 child used a stethoscope to listen to the dog's heartbeat; child collected an AAI-related sticker or card  
19 from the handler; participant used a lint roller during the session; participant brushed the dog; and  
20 participants discussed therapy dogs with the handler (p range = ≤ 0.001 - 0.043).

21 Dogs displayed significantly *more affiliative behaviors per session* when the following activities took  
22 place: participant played with dog's toy; participant talked to the dog; handler discussed dog breeds and  
23 histories with the family; participants took the dog for a walk; participants viewed photos of the dog; and  
24 participants practiced the dog's known cues (p range = ≤ 0.001 - 0.046).

25 Dogs exhibited significantly *fewer stress behaviors per session* when the following activities took place:  
26 child facilitated visit between the therapy dog and hospital staff; participants pet the dog; and participants  
27 took a photo of the dog (p range = ≤ 0.001 - 0.041). Finally, dogs displayed significantly *more stress*

1 *behaviors per session* when the following activities took place: child put a bandanna on the dog and when  
2 the handler and participants discussed dog breeds ( $p = 0.035$  and  $p = 0.003$ , respectively).

### 3 *3.7 Relationship Between Salivary Cortisol and Behavior*

4 The final analysis model included salivary cortisol level as a fixed factor to examine its relationship with  
5 stress and affiliative behaviors. When controlling for affiliative behaviors, higher salivary cortisol was  
6 significantly associated with an increased frequency of stress behaviors per session,  $\beta = 0.16$ ,  $t(243) =$   
7  $2.08$ ,  $p = 0.039$ . When controlling for stress behaviors, higher salivary cortisol was significantly  
8 associated with a reduced frequency of affiliative behaviors,  $\beta = -0.26$ ,  $t(243) = -2.82$ ,  $p = 0.005$ . Thus,  
9 lower levels of salivary cortisol were related to increased displays of affiliative behaviors during AAI  
10 sessions, while higher levels of salivary cortisol were related to increased displays of stress behaviors.

## 11 **4. Discussion**

12 The current study measured physiological and behavioral indicators of stress in registered therapy dogs  
13 who participated in AAI sessions with pediatric oncology patients and their parents. To our knowledge,  
14 this is the largest randomized controlled trial to measure the impact of AAI for both humans and therapy  
15 dogs to date, particularly across multiple hospitals where AAI is commonly practiced. As hypothesized,  
16 results indicate that therapy dogs show minimal signs of distress during AAI sessions, regardless of  
17 hospital site. The geographical heterogeneity (across the U.S.) of participating sites has favorable  
18 implications for the generalizability of these findings, particularly in healthcare facilities with rigorous  
19 therapy dog programming (Linder et al., in press).

20 The lack of significant differences in canine salivary cortisol levels between AAI sessions and each of the  
21 five baseline conditions is an important finding that supports existing selected studies in this area (Glenk  
22 et al., 2013, 2014; Ng et al., 2014). Cortisol concentration levels also remained relatively consistent over  
23 time, which could indicate that participation in a greater number of AAI sessions is not a source of stress  
24 for therapy dogs.

25 Additionally, there was a significant relationship between canine cortisol and behavior, with lower  
26 cortisol levels associated with increased displays of affiliative behavior, and higher cortisol levels with

1 increased stress behaviors. Both of these relationships, but particularly the latter, suggest that cortisol may  
2 be a good indicator of distress in therapy dogs. Notably, there were few significant relationships between  
3 handler-rated canine temperament and canine outcomes, except for the C-BARQ stranger-directed fear  
4 factor; dogs with a perceived higher degree of stranger-directed fear exhibited significantly fewer  
5 affiliative behaviors on average per session. Much like with novel settings (Ng et al., 2014), therapy dogs  
6 who find interactions with strangers stressful may benefit from a prolonged introduction to unfamiliar  
7 recipients, possibly comprised of shorter initial visits where the dog can initiate contact and take breaks  
8 when needed. Further, canine temperament measures like the C-BARQ may serve as effective tools for  
9 facilities when screening prospective therapy dog-handler teams, and determining their optimal  
10 placement.

11 In addition, while therapy dogs exhibited slightly more stress behaviors than affiliative behaviors per  
12 session on average, the difference between the two was not significant and may have been due to more  
13 stress behaviors listed on the ethogram for researchers to code. Dogs who displayed a high frequency of  
14 affiliative behaviors tended to exhibit a similarly high frequency of stress behaviors, which could indicate  
15 greater expressiveness in these dogs.

16 Interestingly, older dogs displayed significantly more stress and affiliative behaviors than their younger  
17 counterparts, but also had significantly lower session cortisol. Potentially, some dogs may grow  
18 increasingly expressive in their behavior or adept at communicating behaviorally as they age. Further,  
19 behaviors traditionally deemed as stress indicators – such as panting or yawning (Beerda et al., 1998,  
20 1999) – could serve as a calming mechanism for dogs to manage their stress during worrisome situations,  
21 as Glenk et al. (2014) propose. Thus, this could lead to a higher number of stress behaviors, but a lower  
22 physiological stress response, as we observed with older therapy dog participants. With that said, it is  
23 essential that handlers be responsive to the changing health needs of their therapy dogs as they age, and  
24 retire dogs when they are no longer able to engage comfortably with recipients (Serpell et al., 2010).

25 Years of handler experience in AAI was not significantly related to either their dog's physiological or  
26 behavioral outcomes; just as many stress indicators were present with more experienced handlers as with

1 those who were newer to the practice. For less seasoned handlers, fewer years of experience could have  
2 been offset by a shorter time lapse since undergoing training. As AAI trainings often emphasize the  
3 primary role of handlers as stewards of their dog's safety and comfort (Pet Partners, 2017), new handlers  
4 may have been more keenly attentive to canine behavior than those more accustomed to the practice. This  
5 finding highlights the importance of regular handler training opportunities that emphasize how to  
6 effectively monitor and alleviate canine stress during sessions. Nevertheless, it can be argued that handler  
7 participants overall were heedful of their dog's needs, as evidenced by the significant relationship  
8 between longer sessions and an increased frequency of affiliative canine behaviors. Handlers who  
9 observed a relatively high frequency of affiliative behaviors in their dogs may have continued or even  
10 prolonged AAI sessions due to the perceived low risk of canine stress. However, given the small effect  
11 size of this relationship, and the large range of documented session duration, this interpretation remains  
12 speculative and needs further exploration.

13 There were several limitations of this study that may serve to clarify areas of future inquiry. While our  
14 canine sample size is the largest known to date in studies of therapy dog welfare (Glenk, 2017), we  
15 studied less than 30 dogs. Subsequent research would benefit from a larger sampling of therapy dog  
16 participants. However, the sample was strengthened by its heterogeneity and representation of the breed  
17 variability currently observed in AAI applications.

18 There were also certain outside factors that could have impacted the level of stress experienced by therapy  
19 dogs that we did not document, including their adoption experiences, trauma histories, or any record of  
20 serious health conditions or medication regimens (although all dogs received prior veterinary approval for  
21 AAI participation). We also did not note characteristics of AAI visits that the dogs may have participated  
22 in between study sessions, such as length, number, frequency, and population served. Indeed, due to  
23 ethical reasons, therapy dogs were not prohibited from visiting with other people that they encountered  
24 when on hospital premises for study sessions, and we did not inquire about these interactions.

25 Additionally, handler participants, while anecdotally representative, were not demographically diverse  
26 enough to explore the potential effects of handler race, gender, or age on therapy dog well-being. Future

1 research and practice efforts should focus on the potential impact of handler demographics on canine and  
2 human recipient outcomes, as well as how this population may be diversified to ensure culturally  
3 competent AAI practices. Furthermore, handler adherence to certain study protocols, such as the timing of  
4 baseline saliva collection, was not consistent, and could have impacted the interpretation of those data.  
5 Further, in order to study the canine effects of AAI sessions as they are currently practiced, this study did  
6 not prescribe a structured protocol of activities. Rather, the flow and type of session activities occurred at  
7 the discretion of patients, parents, handlers, and/or medical staff, and were largely dependent on child  
8 health status and/or the response of the child or dog to the interaction. Activities were documented,  
9 including how frequently they were engaged by both children and their parents.

10 While there were no significant effects of session activities on canine salivary cortisol, there were  
11 significant relationships between certain activities and the frequency of affiliative and stress behaviors.  
12 For example, sessions where the therapy dog was brushed may have been more stressful for the dog, as  
13 evidenced by the presence of less affiliative behaviors. Likewise, dogs displayed more affiliative  
14 behaviors, and potentially less stress, in sessions where they were taken for a walk. However, at present, it  
15 is not sound to infer a direct causal relationship between any particular activity and the dogs' response.  
16 Additionally, the direction of the activity and associated canine behavior is not clear (i.e., did the dog  
17 show more affiliative behaviors when walked or was the dog walked because he/she showed more  
18 affiliative behaviors?). These research questions should be explored further, as they may help to refine  
19 prescribed protocols for future AAI research and practice.

20 Finally, there may have been potential issues regarding the behavior ethogram used in this study. Because  
21 a validated instrument for assessing therapy dog behavior during AAI sessions does not currently exist,  
22 piloting and utilizing our own measure was necessary. In developing our ethogram, it was important to  
23 refer to previous canine behavioral measures (i.e., Beerda et al., 1998), consult with animal behaviorists  
24 and veterinarians, and provide further context through the inclusion of both stress and affiliative  
25 behaviors. Nevertheless, the development of a validated measure which re-evaluates the nature of

1 currently recognized behavioral indicators of therapy dog stress and affiliation in an AAI context is  
2 important to confirm this study’s findings and strengthen those of future, related inquiries.  
3 The increasing prevalence of therapy dog programs in today’s pediatric hospitals and other services  
4 merits further investigation regarding the well-being of dogs who work in these settings (Chubak &  
5 Hawkes, 2016). AAIs have traditionally been positioned as services to improve human health, but the  
6 welfare needs of therapy animals must be elevated if their participation in this pursuit is to advance.  
7 While further research is needed to verify these results, this study provides valuable and rigorous  
8 evidence that AAI participation in pediatric hospitals does not appear to place registered therapy dogs at  
9 significant risk of stress.

10

11 *Funding:* This work was supported by Zoetis; Morris Animal Foundation exclusively from a partnership  
12 with the Human-Animal Bond Research Institute (D14HA-012, 2014); Newman’s Own Foundation; and  
13 the Nora Roberts Foundation. The above funding sources did not have a role in the study design; in the  
14 collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the  
15 article for publication.

16 *Acknowledgments:* The authors wish to thank all the children, families, therapy dogs, handlers, hospitals,  
17 and partners who participated in this study and made this research possible.

18 *Conflicts of interest:* none.

19

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1 **Table 1** Description of the activities during 445 animal-assisted intervention (AAI) sessions

Activities	Child		Parent	
Pet the Dog	409	92%	244	55%
Talk to the Dog	307	69%	212	48%
Viewed Dog's Photos	144	32%	64	14%
Brush the Dog	106	24%	16	4%
Practice the Dog's Cues	99	22%	29	7%
Discussed Dog Breeds	62	14%	55	12%
Discuss Therapy Dogs	56	13%	48	11%
Walk the Dog	53	12%	10	2%
Take Photo of the Dog	43	10%	69	16%
Play with Dog's Toy	42	9%	15	3%
Facilitated Dog's Visit with Staff	24	5%	16	4%
Collected a Sticker from Handler	22	5%	2	0%
Used Lint Roller	18	4%	4	1%
Listened to Dog's Heart Beat	13	3%	3	1%
Taught Dog New Trick	10	2%	5	1%
Colored a Picture	8	2%	0	0%
Put Bandana on the Dog	8	2%	3	1%
Got the Dog Water	6	1%	0	0%
Drew a Picture of or for the Dog	6	1%	0	0%
Read to the Dog	4	1%	3	1%
Played a Game	3	1%	1	0%
Played on Dog's Vest	3	1%	2	0%

2

1 **Table 2** Frequency of behaviors during 405 animal-assisted intervention (AAI) sessions

<i>Behavior</i>	<i>Per Session</i>		<i>Per Minute</i>	
	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Stress	9.69	10.47	0.61	0.59
Affiliative	9.14	9.33	0.59	0.56
Oral Behaviors	4.52	7.28	0.28	0.42
Licking	2.31	3.46	0.15	0.21
Tail Wagging	1.98	3.25	0.13	0.23
Yawning	1.39	1.84	0.09	0.11
Raising Ears	1.24	3.89	0.07	0.21
Panting	1.17	1.36	0.08	0.10
Snout	1.09	1.67	0.07	0.12
Leaning	0.97	1.71	0.06	0.10
Lifting Paw	0.97	2.13	0.06	0.14
Look at Handler	0.79	1.32	0.05	0.09
Shaking	0.79	1.21	0.05	0.08
Walking	0.46	0.81	0.03	0.06
Restlessness	0.29	1.25	0.02	0.07
Look Away	0.26	1.16	0.02	0.07
Self-Directed Behaviors	0.25	0.78	0.02	0.05
Escape	0.21	0.82	0.01	0.04
Roll Over	0.09	0.42	0.01	0.04
Play: Bow Stance	0.03	0.19	0.00	0.01
Vocalization	0.01	0.11	0.00	0.01
Bite	0.00	0.00	0.00	0.00
Crouching	0.00	0.00	0.00	0.00
Drooling	0.00	0.00	0.00	0.00
Ears Pinned Back	0.00	0.05	0.00	0.00
Growling	0.00	0.00	0.00	0.00
Stare	0.00	0.00	0.00	0.00
Teeth	0.00	0.05	0.00	0.00

2

3 **Table 3** Salivary cortisol across conditions ( $N = 411$  samples, 24 dogs)

<i>Condition</i>	<u>b</u>	<u>SE</u>
Baseline – Morning	-0.05	0.06
Baseline - Mid-Day	0.02	0.06
Baseline – Evening	0.08	0.08
Baseline – Trigger	-0.04	0.06
Baseline – Hospital	0.03	0.06

4 Note: Reference category (AAI sessions), \* $p \leq 0.05$ , \*\* $p \leq 0.01$ .

1 **Table 4** Relationship between demographic characteristics and salivary cortisol (N = 406 samples, 23  
 2 dogs)

<i>Demographic Characteristic</i>	<i>Cortisol</i>		<i>Stress Behaviors</i>		<i>Affiliative Behaviors</i>	
	<u>B</u>	<u>SE</u>	<u>b</u>	<u>SE</u>	<u>b</u>	<u>SE</u>
Dog Gender (Female vs Male)	-0.28**	0.09	-0.16	0.17	0.05	0.25
Dog Age (Years)	-0.05*	0.02	0.15**	0.03	0.14**	0.04
Handler Experience (Years)	-0.01	0.01	-0.04	0.03	0.06	0.04
Breed (Lab/Golden vs Other)	0.25*	0.09	-0.22	0.17	0.70**	0.25

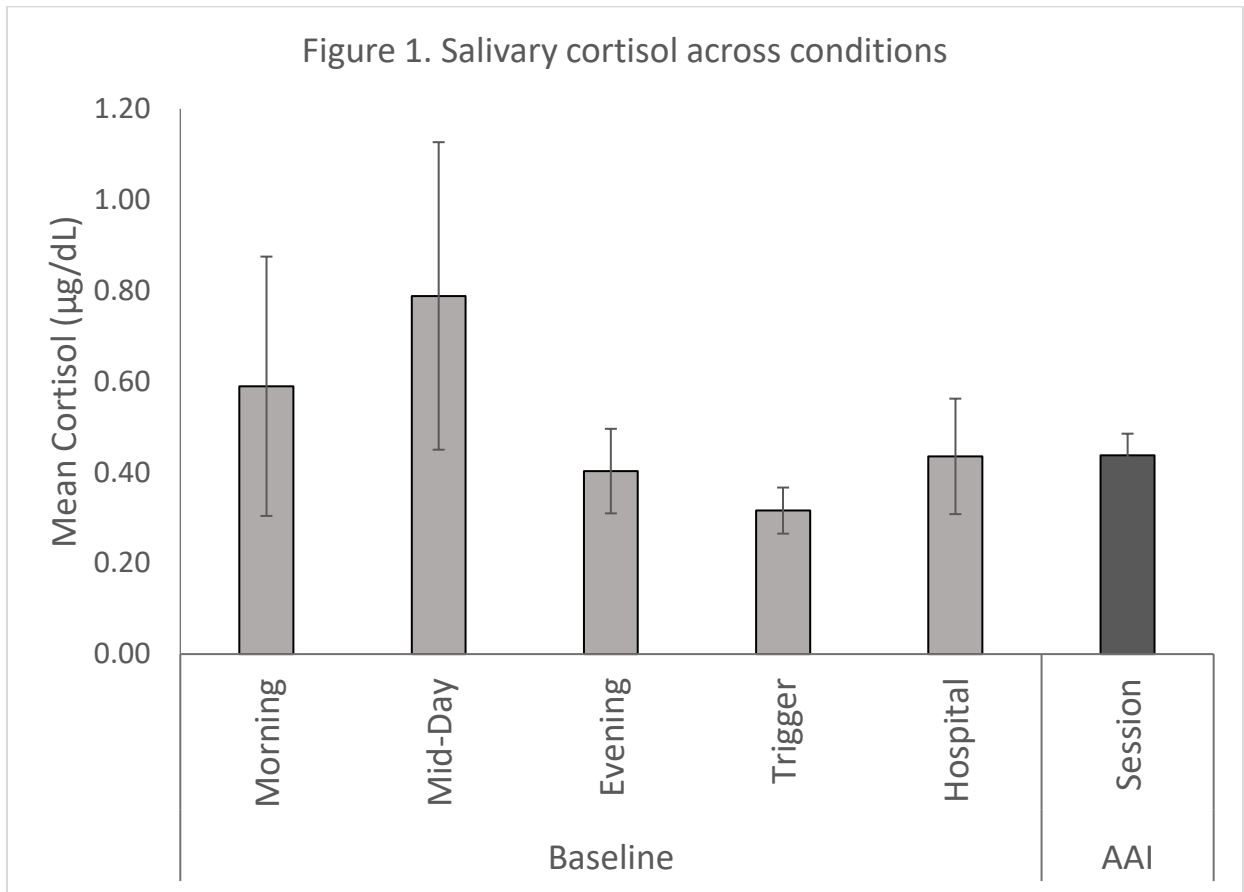
3 Note: \*p ≤ 0.05, \*\*p ≤ 0.01.

4  
 5 **Table 5** Relationship between session characteristics and salivary cortisol (N = 254 samples, 23 dogs)  
 6 and ethogram stress and affiliative behavior (N = 368 samples, 25 dogs)

<i>Session Characteristic</i>	<i>Cortisol</i>		<i>Stress Behaviors</i>		<i>Affiliative Behaviors</i>	
	<u>B</u>	<u>SE</u>	<u>b</u>	<u>SE</u>	<u>b</u>	<u>SE</u>
Length of Session	0.00	0.00	0.00	0.00	0.01**	0.00
Number of people present	-0.03	0.02	-0.02	0.02	0.04	0.02
Number of people participating	0.03	0.02	-0.01	0.02	0.03	0.02

7 Note: \*p ≤ 0.05, \*\*p ≤ 0.01.

1 **Figure 1** Salivary cortisol concentrations across conditions



2

1 **Figure 2** Relationship between session activities and ethogram stress and affiliative behaviors (N = 368  
 2 samples, 25 dogs)

+ Affiliative Behaviors	+ Stress Behaviors
Played with dog's toy (b = 0.47; p < .0001. Talked to the dog (b = 0.26; p < .0001) Discussed dog breeds and histories (b= 0.16; p = .002) Took the dog for a walk (b = 0.14; p = .046) Viewed photos of the dog (b= 0.13; p = .006). Practiced the dog's known cues (b = 0.11; p= .033).	Put a bandanna on the dog (b = 0.26; p = .035) Discussed dog breeds and histories (b = 0.15, p = .003).
- Affiliative Behaviors	- Stress Behaviors
Brushed the dog (b = 0.15; p = .003) Got water for the dog (b = 1.32; p = 0.006) Used a lint roller (b = .20; p = .030) Drew a picture of the dog (b = 0.45; p = 0.003) Listened to the dog's heart beat (b = 0.33; p = 0.004) Collected therapy dog sticker (b = 0.23; p = 0.043) Played dog board or card game (b = 1.00; p < 0.001) Discussed therapy dogs (b = 0.14; p = 0.020)	Facilitated visit between dog and staff (b = 0.45; p < .0001) Pet the dog (b = 0.17; p = .034) Took a photo of the dog (b = 0.10; p = .041).

3