

Comparison between Aqua-acupuncture and Trazodone on Mitigation of Fear, Anxiety and Stress in Healthy Dogs during Routine Veterinary Examination

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ABSTRACT

Fear, anxiety and stress (FAS) are significant factors in human-animal interactions in veterinary hospital settings. The objective of this study was to determine the effect of aqua-acupuncture (Aqua-AP) compared with trazodone on the mitigation of FAS indicators in healthy, stressed dogs during routine veterinary examination and procedures. Fifty-five dogs exhibiting signs of FAS or history of significant FAS in veterinary hospital settings completed the study. Except for those premedicated with trazodone (Group 4), dogs were randomly assigned to 3 treatment groups. Groups 1 (*An-shen*) and 2 (GB-21) were treated with Aqua-AP bilaterally, while Group 3 served as untreated controls. Stress data, including heart rate, panting respiratory rate, behavior and saliva cortisol concentration were taken at T₀ (baseline) and T₃₀ (30 minutes post-treatment). Comparison of group outcome data found that Aqua-AP groups had significant decreases for heart rate ($p = 0.004$ and $p = 0.0005$, respectively) and behavior score ($p = 0.0002$ and $p = 0.008$, respectively) from T₀ to T₃₀, and post-treatment values were not higher than the pharmaceutical-treated subjects (noninferiority). In addition, Group 1 had significant reduction ($p = 0.04$) in incidence of panting respiratory rate. No significant stress parameter changes were observed in controls. The cortisol concentration change was not significant in any group, but the post-treatment cortisol concentration in both Aqua-AP groups was not greater than the trazodone group ($p = 0.004$ and $p = 0.018$, respectively). These results suggest that Aqua-AP at *An-shen* or GB-21 is effective in mitigating FAS in healthy dogs during routine veterinary examination and demonstrates noninferiority to premedication with trazodone.

Keywords: FAS, aqua-acupuncture, trazodone, *An-shen*, GB-21, canine anxiety, fear, stress

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ABBREVIATIONS

AP	Acupuncture
Aqua-AP	Aqua-acupuncture
DNAP	Dry needle acupuncture
FAS	Fear, anxiety, stress
HR	Heart rate
NSAID	Nonsteroidal anti-inflammatory drug
RR	Respiratory rate
TCM	Traditional Chinese medicine
TCVM	Traditional Chinese veterinary medicine

Fear, anxiety and stress (referred to as FAS) are common and significant complicating factors in human animal interactions in veterinary medicine.¹ A recent study explored the risk factors associated with fear during veterinary examination from 26,555 owner responses in a behavioral questionnaire database. It was found that 41% of companion dogs displayed mild to moderate fearful

behavior when examined by a veterinarian, and 14% exhibited severe or extreme fear.² Veterinarians are thus faced with the challenge of trying to measure changing physical parameters dependent on an animal's mental state as well as carrying out important medical procedures on patients that may be under extreme stress.

When considering the magnitude of this problem, consideration of what happens physically to an animal undergoing a stressful experience (stressor) is important. First, a stress response occurs, which is defined as the physiologic and behavioral changes that attempt to return the body to a normal state of function. This response can be mild or intense along a spectrum from a quick return to baseline or escalation to extreme stress, defined as distress.¹ Various body systems can be affected by the immediate stress response. Heart and respiratory rates increase, blood circulation is diverted to the muscles, blood glucose levels increase and the animal experiences decreased pain perception to facilitate escape from the stressor.³⁻⁴ There is a decrease in rational thoughts and

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increase in memory consolidation and retrieval.⁴⁻⁵ The immediate stress response occurs within less than a second of presentation of a stimulus. Evaluation and quantification of stress in dogs can include assessment of body language and posture as well as cortisol measurements. Panting, pacing, lip smacking, tucking the tail and pulling the ears back are all behavioral markers of stress in dogs. Panting and lip smacking have conclusively been linked to elevated cortisol levels in dogs in hospitalized situations.⁶

A strong emphasis on improving the emotional and physical experiences of dogs in a veterinary hospital setting has been made over recent years. Measures include raising public awareness of FAS and teaching strategies to the veterinary team and owners on how to prevent and alleviate stress responses.¹ Pheromone products, pre-visit pharmaceuticals and or natural supplements to help calm the patient can be applied. Species appropriate handling with positive reinforcement or luring with high value treats or toys are additional options.¹ Music therapy has been associated with positive outcome and long-term stress reduction in humans on maintenance hemodialysis for the management of chronic renal disease.⁷ Once behavior modification techniques, conditioning and the use of a considerate approach to the animal have been exhausted in cases with moderate to severe FAS, in-hospital sedation remains the only viable approach to facilitate necessary procedures (e.g., examination, nail trims, diagnostic procedures). Medications commonly used include benzodiazepines, selective serotonin re-uptake inhibitors (SSRIs), alpha-2 agonists and morphine derivatives, all of which carry specific risks and side effects (e.g., ataxia, drop in blood pressure, nausea, idiosyncratic reactions).⁸

In traditional Chinese veterinary medicine (TCVM), FAS is considered an imbalance of the Fire Element and described as *Shen* disturbance. *Shen* refers to the outward appearance of the vital activities of the whole body. It rules the mind, mental activities, memory and sleep. It provides awareness and clear feeling. A healthy *Shen* equals inner peace. An animal with healthy *Shen* will exhibit normal, calm behaviors and will be alert and responsive to environmental stimuli. *Shen* is housed in the Heart and requires nourishment with Heart *Yin* and Blood.⁹ The acute presentation of FAS correlates to the Excess TCVM pattern of Excessive Fire damaging Heart *Yin* and Blood. Clinical signs include restlessness, palpitations/increased heart rate, anxiety, being easily frightened and unable to focus attention.

Methods in traditional Chinese medicine (TCM) for treating *Shen* disturbance include acupuncture (AP), Chinese herbal medicine, nutritional changes, and mindfulness exercises including traditional *Tai Chi* and *Qi Gong*. Numerous clinical studies have been conducted demonstrating the effects of AP in both acute and chronic stress.¹⁰⁻¹⁶ Acupuncture, which involves the insertion and retention of a specialized AP needle into an AP point, requires cooperation and tolerance by the patient. As

much as humans can provide informed consent, canine patients, especially when exposed to stress factors (i.e., veterinary clinic), lack such understanding.

Dry needle acupuncture (DNAP) may be considered to pose an additional stress factor. Complications may include repetitive needle insertions within a short period of time due to inability of a patient to hold still for the commonly used 10-20 minute duration of needle retention. On the other hand, aqua-acupuncture (Aqua-AP) is a stimulation technique that uses injection of a liquid substance at an AP point. It combines acupoint stimulation with the therapeutic/pharmacological benefit of the injected substance. It has been demonstrated that the spatial configuration changes of the acupoint activate neuronal signaling and enhance stimulation when compared to DNAP.¹⁷ In addition, the type of liquid substrate injected, may further strengthen the neuronal activity.¹⁷ Single point Aqua-AP, therefore, may serve as a simple and easily teachable technique for veterinary professionals faced with FAS in the exam room. It involves both minimal manipulation of the patient and easily available supplies. All that is needed are a syringe, hypodermic needles and injectable vitamin B₁₂ or normal saline. The use of Aqua-AP can be accomplished quickly, eliminating potential side effects of sedating or anxiolytic pharmaceuticals and may aid in reduction of FAS.

Two AP points that provide sedation effects (*An-shen*, GB-21) are proposed as effective for FAS mitigation. *An-shen* is a classical acupuncture point located at the ear base in a deep depression caudal to the skull and has been suggested as a calming point in TCVM.¹⁸ Gallbladder 21 (GB-21) is located halfway between the dorsal midline at C7/T1 and the acromion alongside the scapula and is commonly used in TCM for stress reduction.¹⁹ Both *An-shen* and GB-21 have additional benefits as they are easily accessible, even in animals with moderate to severe FAS, which may require muzzling.

A recent study demonstrated beneficial effects of Aqua-AP at both GV-20 and bilateral *An-shen* for mitigating veterinary clinic stress in dogs but no comparison was made to current common pre-medications used to treat FAS.²⁰ The present study proposed investigating the use of Aqua-AP at bilateral GB-21 and bilateral *An-shen*, with comparison to untreated controls as well as a group that was premedicated with trazodone at least 2 hours prior to veterinary exam. The objective was to determine whether the 2 Aqua-AP test groups had mitigating effects on FAS indicators, and that the treatment was at least as effective as trazodone. The study hypothesis was that dogs affected with veterinary visit FAS treated with Aqua-AP at sedation acupoints (*An-shen*, GB-21) would demonstrate greater reduction in FAS clinical signs than untreated controls and would demonstrate noninferiority to dogs premedicated with the serotonin modulator, trazadone.

MATERIALS AND METHODS

The subject population for this study consisted of healthy dogs presenting for routine veterinary wellness exams and showing demonstrative signs of FAS or having a history of significant FAS in the veterinary hospital setting. Common symptoms of FAS include pacing, panting, lip smacking and vocalization. All study subjects were recruited at the author's small animal hospital, K.Vet Animal Care, located in Greensburg, Pennsylvania, USA. Owners were advised about and invited to participate in the clinical study upon check-in at the reception desk. All owners were handed a standardized consent and liability form explaining the procedures and goals of the study, available in hard copy form at the hospital as well as on the hospital website. The owners were also advised that all study related procedures (Aqua-AP, saliva cortisol analyses) were no charge to the owner. Subject inclusion criteria were: (1) age > 1 year old; and (2) body weight greater than 40 lbs. Smaller dogs were expected to experience more stress during extended periods of saliva collection and have been documented to produce insufficient quantities of saliva for cortisol measurement.²⁰ Only gonadectomized individuals were chosen for this study to eliminate hormonal influences on the hypothalamus-pituitary-adrenal (HPA) axis as demonstrated in humans.²¹ The definition of healthy included absence of chronic metabolic diseases (e.g., Cushing's disease, diabetes mellitus, hypothyroidism, Addison's disease). In addition, patients with any of the following conditions were excluded from the study: (1) on long-term nonsteroidal anti-inflammatory drugs (NSAIDS) or pain medications for the management of chronic arthritis or other pain conditions; (2) seizure disorders and on chronic anti-epileptic medications; (3) acute illness requiring medications (e.g., antibiotics, NSAIDS) within 2 weeks prior to the wellness exam; and (4) recovering from surgery within 2 weeks of the wellness exam.

Qualified subjects were allocated into 4 treatment groups: Group 1 received Aqua-AP at *An-shen*, bilaterally; Group 2 received Aqua-AP at GB-21, bilaterally; Group 3 (controls) received no premedication and no Aqua-AP or Group 4 which was orally premedicated with trazodone^a (4mg/kg) at least 2 hours prior to exam and no Aqua-AP. Only dogs with a pre-existing history of FAS and an active trazodone pre-veterinary visit prescription were included in Group 4. Dogs that were not premedicated with trazodone (i.e., not in Group 4) were randomly assigned to the other 3 groups via pull bag sample randomization by a staff member.

Upon presentation to the hospital for their routine wellness appointment each potential study dog's owner was given information on the study and if interested was directed to the waiting room where medical history and study participation paperwork was filled out. The enrolled dog and owner were then brought to an exam room, no longer than 20 minutes after they had entered the waiting room area. The dog was observed by the wellness team

technician for signs of FAS (pacing, panting, lip smacking, vocalization, hiding behavior). If the dog exhibited clinical signs of FAS or if the dog was premedicated with trazodone due to past FAS, the technician scored the FAS signs (absent = 0 or present = 1) and the dog was considered enrolled in the study. The technician also measured baseline (T_0) heart rate (HR), respiratory rate (RR) and collected a saliva sample at this time.

To collect saliva, the dogs were teased with a high reward treat (peanut butter pretzel or favorite treat from home) to enhance production and rewarded afterwards with said treat. The saliva sample was collected by holding a cotton dental rope^b measuring approximately 3 inches in the dog's mouth for approximately one minute. The saliva saturated part of the rope was then cut off and placed into a 5 cc syringe^c and compressed to extract the saliva into a 2 cc freezable vial^b. Citric acid administration was avoided prior to saliva collection, as it has been demonstrated to influence saliva cortisol concentrations in a previous study.⁶ All samples were kept frozen at -18°C until being processed. The time frame from entering the hospital setting to first saliva sample collection was approximately 20 minutes to normalize for wait times and take advantage of canine saliva cortisol level peaking at 20 minutes after initiation of stressful stimuli.^{22,23}

Following collection of the initial data (baseline, T_0), the room technician would leave the exam room to collect the randomly assigned group number 1-3 (pull bag sample randomization) by a staff member and inform the doctor about it. The veterinarian to perform the wellness exam and room technician then entered the exam room together. Aqua-acupuncture was performed right away by the veterinarian if indicated. For all Aqua-AP subjects (Group 1, Group 2), a full 3 cc syringe with an attached 25 gauge needle^c of vitamin B₁₂ (1000 ug/ml)^d was provided to the treating veterinarian, with the goal to sufficiently distend the anatomical area with fluid. If a dog moved or showed discomfort, then only 1 cc was instilled in an acupoint, whereas, if allowed by the dog, the full amount was injected. On average, 1.0-1.5 cc of vitamin B₁₂ per AP point was applied bilaterally into *An-shen* (Group 1) or into GB-21 (Group 2). *An-shen* is located in a deep depression caudal to the ear base. Gallbladder 21 is a meridian point located halfway between two other acupuncture points at the proximal aspect of the scapula (Governing Vessel 14) and the distal aspect of the acromion (Large Intestine 15).

The veterinarian then performed the wellness physical exam. For all additional procedures performed, which varied depending on the dog (e.g., vaccination, blood sample collection, fecals, urine, nail trims, anal sac expression), the animal was taken to the treatment room where the procedures were performed by treatment area technicians who were unaware of study groups. At exactly 30 minutes (T_{30}) after the initial (baseline, T_0) data was collected, a second saliva sample was obtained by whichever technician, at that point, was attending the

pet, along with FAS, HR, and RR measurements, independent of completion status of the other wellness procedures.

After the 2 saliva samples (T_0 and T_{30}) had been collected from all participating study dogs, the frozen samples were thawed, centrifuged^e for 15 minutes at 3000 rpm to remove mucins before sample submission for cortisol analysis^b. All samples were measured in duplicate, unless saliva volume prevented this step, and results were averaged for use in statistical analyses. A highly sensitive immunoassay kit^b was used for cortisol analysis for all samples. The test used 25 μ l of saliva and had a range of sensitivity from 0.0007 to 1.8 μ g/dl, with average intra- and inter-assay coefficients of variations less than 10 and 15%, respectively. Method accuracy, determined by spike recovery and linearity, determined by serial dilution, were 105% and 95%, respectively. All samples were sent to the commercial laboratory^b, which supplied the test materials as well as performed all analysis and were blinded to experimental study groups.

An FAS score was assigned (0=absent, 1=present) for each of 5 behaviors (pacing, panting, lip smacking, vocalization, hiding behavior) at both baseline (T_0) and 30 minutes later (T_{30}). A dog's total FAS score at each timepoint, therefore, could range from 0 (no FAS) to 5 (all behaviors present). The study compared FAS measurement changes (T_0 to T_{30}) between the Aqua-AP groups and the untreated controls, as well as compared the post-treatment (T_{30}) outcomes of the Aqua-AP groups with the trazodone-treated T_0 data. This was considered post-treatment because the trazodone treated subjects were premedicated. For each of the FAS indicators, statistical evaluation tested the hypotheses that an Aqua-AP group (Group 1 or Group 2) had a greater reduction (from T_0 to T_{30}) than the no-treatment group (Group 3), and that the FAS indicator at T_{30} was not larger than those receiving trazodone treatment (Group 4).

A non-parametric Wilcoxon rank-sum test was used to determine whether Aqua-AP was more effective than no treatment for study outcome data (cortisol, HR, RR, FAS). To statistically determine whether Aqua-AP was at least as effective as trazodone treatment, the inference was based on 95% confidence interval of the group difference (based on Wilcoxon rank-sum tests). If the lower limit of the confidence interval was greater than d

(d = pre-set non-inferiority margin), the non-inferiority could be established. The pre-set non-inferiority parameter was defined as 0.3 μ g/dl, which is 10% of the expected normal cortisol level (3 μ g/dl) in dogs.⁶

The study design determined that a sample size ($n = 17$) in groups 1 and 2 would provide at least 90% power to reject the null hypothesis ($p = 0.05$ significance level) when the experimental group (i.e., Group 1 or Group 2) had an 80% probability better than the control group (i.e., Group 3, $n=14$). For comparisons between Aqua-AP and trazodone treatment, the sample size would ensure a 95% power to reject the null hypothesis ($p = 0.05$), assuming that the mean in Group 4 was 3.0 μ g/dl, and Group 1 (or 2) had a mean of 2.5 (SD = 0.5). A commercial statistical software was used for all data graphic presentations and statistical analysis^f.

RESULTS

Subjects and Characteristics Data

A total of 59 dogs admitted to K. Vet Animal Care for routine veterinary wellness exams met the inclusion/exclusion criteria and were enrolled in the study. Eight dogs were premedicated at home with trazodone, and the remaining 51 dogs were randomly assigned to Groups 1, 2 and 3, which resulted in 18 dogs in Group 1, 18 in Group 2 and 15 in Group 3. One subject in each group did not complete the trial. There were, therefore, 17 subjects in Groups 1 and 2, 14 in Group 3, and 7 in Group 4 for a total of 55 dogs included in the statistical analysis. The dropouts slightly reduced the study power to 86% for comparisons with Group 3 and reduced to 92% for comparisons with Group 4. No significant differences among groups were observed on sex proportion ($p = 0.416$) and weight ($p = 0.990$), but a significant difference existed for age ($p = 0.009$), which was detected between Group 2 and Group 3. With respect to a subject's breed, Group 1 included 7 dogs of mixed breed, 2 Golden Retrievers and 8 other breeds (1 dog per breed). Group 2 had 3 dogs of mixed breed, 3 Golden Retrievers and 11 other breeds (1 dog/breed). Group 3 had 2 dogs of mixed breed, 6 Golden Retrievers and 6 other breeds (1 dog/breed). Group 4 also had 2 dogs of mixed breed, 2 Golden Retrievers and 3 other breeds (Table 1).

Table 1: Summary of study dog signalment data

	Group 1 (n = 17)	Group 2 (n = 17)	Group 3 (n = 14)	Group 4 (n = 7)	p-value
Sex (F/M)	41.2% / 58.8%	52.9% / 47.1%	42.9% / 57.1%	14.3% / 85.7%	0.416
Age (years)	6.9 \pm 3.6 ^	4.8 \pm 2.1 ^	8.1 \pm 2.5 ^	7.1 \pm 1.9 ^	0.009**
Weight (lbs.)	66.1 \pm 17.1 ^	66.5 \pm 20.9 ^	65.8 \pm 13.5 ^	69.3 \pm 27.5 ^	0.990
Breed Totals	10 + mixed (7)	14 + mixed (3)	12 + mixed (2)	5 + mixed (2)	

* = statistical significance $p < 0.05$, ** = statistical significance $p < 0.01$; ^ = mean \pm SD

Heart Rate

Heart rates were measured approximately 20 minutes after arrival (T_0) and 30 minutes after the veterinary examination was started (T_{30}). Groups 1 and 2 had statistically significant mean reductions of HR ($p=0.004$ and $p=0.0005$, respectively) while both the controls and Group 4 did not ($p = 0.22$ and $p = 0.50$) (Table 2, Figure 1). Compared with the decrease in the controls, however, neither Aqua-AP treated group or the combination of the 2 groups had a large enough HR reduction to show a statistically significant difference from the controls. Both Aqua-AP treated groups were also compared to the trazodone treated group for non-inferiority. When compared to Group 4, both Aqua-AP groups' HRs were at least as low as the premedicated group's score with a noninferiority margin of 5 per minute at T_0 (Group 1 $p = 0.02$ and Group 2 $p = 0.03$) and T_{30} (Group 1 $p = 0.01$ and Group 2 $p = 0.04$).

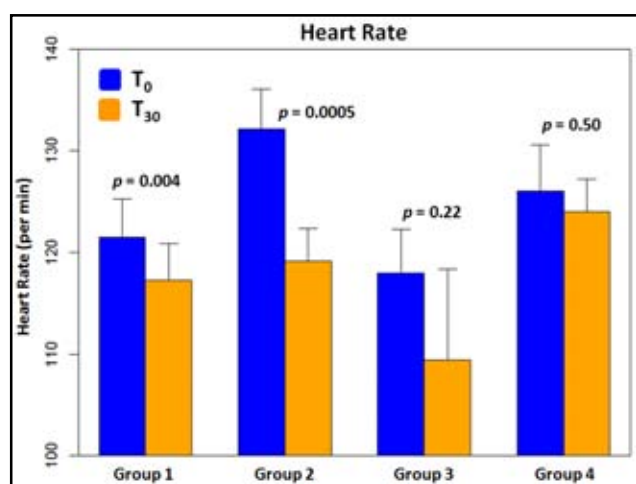


Figure 1: Heart rate at the initial sampling time (T_0 - blue bars) and 30 minutes after T_0 (T_{30} - orange bars) in each subject group; both Aqua-AP groups (groups 1 and 2) demonstrate a statistically significant reduction in heart rates versus no statistical significance for controls and premedicated group (groups 3 and 4).

Respiratory Rate

Group 1 had a statistically significant reduction of proportion of dogs with a panting RR ($p = 0.04$) between T_0 and T_{30} , while the other 3 groups had non-statistically significant ($p = 1.00$) reductions (Table 3, Figure 2). Among the 11 Group 1 subjects with panting RR at T_0 , 6 did not exhibit panting RR after the Aqua-AP treatment (resolving rate = 55%), whereas among the 8 Group 3 dogs (controls) with panting RR at T_0 , only 1 did not exhibit panting RR at T_{30} (resolving rate = 13%). The statistically significant reduction of panting RR in Group 1, however, was not of sufficient magnitude in this small study to claim a significantly larger resolving rate in Group 1 over Group 3 ($p = 0.08$) with 95% confidence. When compared with the trazodone-treated group for post-treatment panting RR proportions, with a non-inferiority margin of 5%, Group 1 (*An-shen*) was at least as small as that in Group 4 (T_0 : $p = 5.9 \times 10^{-9}$; T_{30} : $p = 0.012$). The proportion in Group 2 (GB-21) was also at least as small as that in Group 4 at T_0 ($p = 0.004$) but could not conclude non-inferiority to Group 4 at T_{30} ($p = 0.346$).

Behavior Score

The overall behavior score (pacing + panting + lip smacking + vocalization + hiding) was scored with a possible range of best score = 0 for a dog (none of the behaviors present) to the most severe score = 5 (a dog exhibited all 5 behaviors). Group mean scores had a statistically significant reduction from T_0 to T_{30} in both Group 1 ($p = 0.0002$) and Group 2 ($p = 0.008$) (Table 4, Figure 3). Behavior mean scores did not have a statistically significant reduction in the Control Group ($p = 0.051$). Aqua-AP mean scores were compared to the control mean; however, in this small study the score reduction was not large enough to achieve statistical significance when compared to control. When compared to Group 4, both Aqua-AP groups' (either T_0 or T_{30}) behavior scores were at least as low as the premedicated group's score with a noninferiority margin of 0.5 at T_0 ($p = 9.9 \times 10^{-6}$ Group 1, and 5.0×10^{-5} Group 2) and T_{30} (2.0×10^{-5} Group 1 and 0.0007 Group 2).

Table 2: Mean heart rate results T_0 to T_{30} for each study group with group comparison and statistical significance

Heart Rate	Group 1 <i>An-shen</i>	Group 2 GB-21	Group 3 Controls	Group 4 Trazodone
T_0 (mean \pm SD)	121.5 \pm 15.6	132.2 \pm 15.9	118.0 \pm 16.1	126.0 \pm 11.3
T_{30} (mean \pm SD)	117.3 \pm 14.8	119.1 \pm 13.4	109.4 \pm 33.4	124.0 \pm 7.8
Difference	4.18 \pm 4.8 $p = 0.004^{**}$	13.1 \pm 12.3 $p = 0.0005^{**}$	8.6 \pm 32.3 $p = 0.22$	2.0 \pm 5.9 $p = 0.50$
% Change (Improvement)	3.4%	9.9%	7.3%	1.6%
Non-inferiority to Group 4	T_0 $p = 0.02^*$ T_{30} $p = 0.01^*$	T_0 $p = 0.03^*$ T_{30} $p = 0.04^*$		

* = statistical significance $p < 0.05$, ** = statistical significance $p < 0.01$

Table 3: Summary table of the proportion of subjects with respiratory rate (RR) exhibited as panting (RR > 60 per minute) for each study group at T₀ and T₃₀ with group comparison and statistical significance

Respiratory Rate	Group 1 <i>An-shen</i>	Group 2 GB-21	Group 3 Controls	Group 4 Trazodone
T ₀	11/17 (64.7%)	12/17 (71%)	8/14 (57%)	6/6 (100%)
T ₃₀	5/17 (29.4%)	10/17 (59%)	7/14 (50%)	5/6 (83%)
% Change (Improvement)	35.3% $p = 0.04^*$	11.8% $p = 1.00$	7.1% $p = 1.00$	16.7% $p = 1.00$
Resolution Rate (No panting T ₃₀)	6/11 (54.5%)	2/12 (16.7%)	1/8 (12.5%)	1/6 (16.7%)
Non-inferiority to Group 4	T ₀ $p = 5.9 \times 10^{-9}^{**}$ T ₃₀ $p = 0.012^*$	T ₀ $p = 0.004^{**}$ T ₃₀ $p = 0.346$		

* = statistical significance $p < 0.05$, ** = statistical significance $p < 0.01$ **Table 4:** Summary table of the overall mean±SD behavior scores (pacing + panting + lip smacking + vocalization + hiding) for each study group at T₀ and T₃₀ with group comparison and statistical significance

Behavior Score	Group 1 <i>An-shen</i>	Group 2 GB-21	Group 3 Controls	Group 4 Trazodone
T ₀ (mean±SD)	2.59±0.62	2.71±0.77	2.14±0.95	3.50±0.84
T ₃₀ (mean±SD)	1.53±0.80	2.0±1.06	1.43±1.22	3.17±0.98
Difference	1.06±0.75 $p = 0.0002^{**}$	0.71±0.92 $p = 0.008^{**}$	0.71±1.07 $p = 0.051$	0.33±0.52 $p = 0.50$
% Change (Improvement)	40.9%	26.2%	33.2%	9.4%
Non-inferiority to Group 4	T ₀ $p = 9.9 \times 10^{-6}^{**}$ T ₃₀ $p = 2.0 \times 10^{-5}^{**}$	T ₀ $p = 5.0 \times 10^{-5}^{**}$ T ₃₀ $p = 0.0007^{**}$		

* = statistical significance $p < 0.05$, ** = statistical significance $p < 0.01$ **Table 5:** Saliva cortisol concentration for each study group at T₀ and T₃₀ with group comparison and statistical significance

Saliva Cortisol Concentration	Group 1 <i>An-shen</i>	Group 2 GB-21	Group 3 Controls	Group 4 Trazodone
T ₀ (mean±SD)	0.47±0.28	0.80±0.94	0.60±0.66	1.02±0.94
T ₃₀ (mean±SD)	0.57±0.28	0.72±0.78	0.58±0.58	1.28±1.50
Difference	0.10±0.30 $p = 0.21$	0.09±0.38 $p = 0.33$	0.02±0.70 $p = 0.68$	0.26±1.04 $p = 1.00$
% Change	↑ 21.3%	↓ 11.3%	↓ 3.3%	↑ 25.5%
Non-inferiority to Group 4	T ₀ $p = 0.004^{**}$ T ₃₀ $p = 0.020^*$	T ₀ $p = 0.018^*$ T ₃₀ $p = 0.024^*$		

* = statistical significance $p < 0.05$, ** = statistical significance $p < 0.01$

Saliva Cortisol Concentration

For each subject, saliva samples were collected at the beginning of the study (~ 20 minutes after the arrival, T₀) and 30 minutes after the study started (T₃₀). Over the T₀ to T₃₀ time period, Group 1 demonstrated a slight increase in cortisol concentration while Group 2 had a slight cortisol decrease. Neither Aqua-AP group had statistically significant change between T₀ and T₃₀ (Table 5). Similarly, both the control and premedicated groups did not have statistically significant changes with

the controls demonstrating a slight decrease and the trazadone group an increase of cortisol concentrations. Interestingly, the trazadone medicated group had a much higher cortisol concentration than the other 3 groups for both T₀ and T₃₀ (Table 5). When compared to Group 4, both Aqua-AP groups' (either T₀ or T₃₀) cortisol concentrations were at least as low as the premedicated group's score with a noninferiority margin of 0.3 at T₀ (Group 1 $p = 0.004$, Group 2 $p = 0.018$) and T₃₀ (Group 1 $p = 0.020$, Group 2 $p = 0.024$).

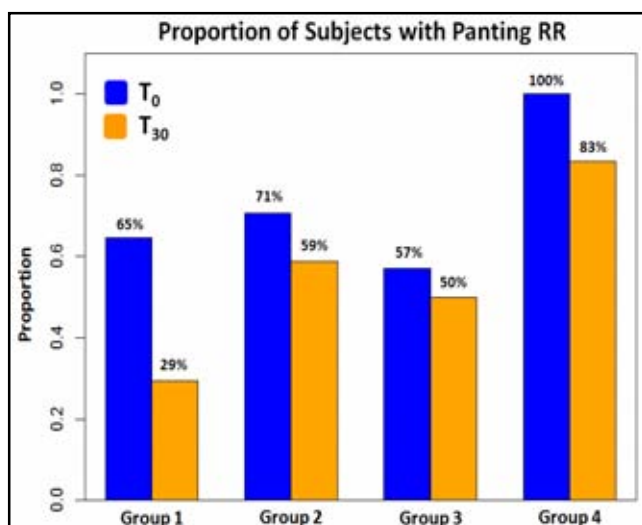


Figure 2: Proportion of subjects with panting RR at the initial sampling time (T₀ - blue bars) and 30 minutes after T₀ (T₃₀ - orange bars) in each subject group; Group 1 (*An-shen* Aqua-AP) had a statistically significant reduction (55% resolving rate) for panting respiratory rate versus the controls with a 13% resolving rate (not statistically significant).

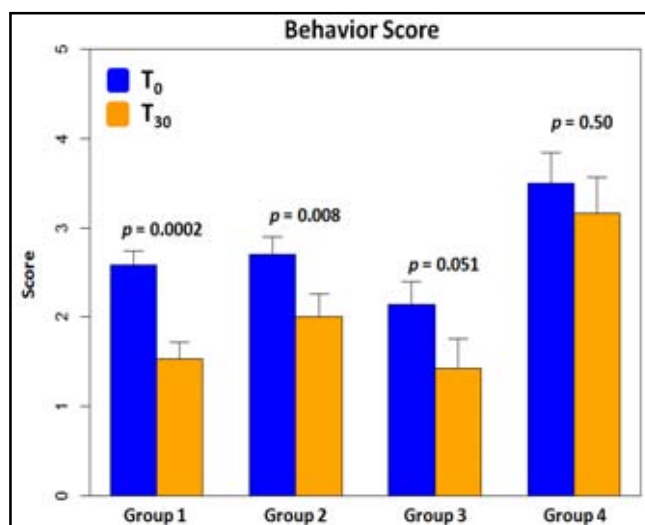


Figure 3: Behavior score at the initial sampling time (T₀ - blue bars) and 30 minutes after T₀ (T₃₀ - orange bars) in each subject group; both Aqua-AP groups (groups 1 and 2) demonstrate a statistically significant reduction in FAS behaviors versus no statistical significance for controls and premedicated group (groups 3 and 4).

DISCUSSION

Veterinarians know that FAS in patients can negatively impact the physical and emotional wellbeing of pets, can alter physiological and laboratory findings and affects the delivery of quality veterinary health care. This controlled, blinded clinical trial had a total of 55 dogs with demonstrative FAS behaviors in veterinary clinical settings complete the study. Collection of FAS measurement changes (heart rate, respiratory rate, behavior, cortisol saliva concentration) from baseline time (T₀) to 30 minutes post treatment (T₃₀) in study dogs was performed. The objective of the study was to compare treatment of FAS with Aqua-AP at sedating acupoints (*An-shen* or GB-21) to untreated controls and dogs premedicated with the serotonin modulator, trazodone. The study demonstrated statistically significant reductions in HR and behavior scores for both Aqua-AP groups with *An-shen* also having a statistically significant reduction in respiratory rate. There were no statistically significant findings in both the untreated controls and trazodone medicated group. In addition, there was a statistically significant demonstration of non-inferiority of the Aqua-AP groups compared to the premedicated group's baseline for all FAS parameters (HR, RR, behavior), including saliva cortisol concentration. These findings supported the study hypothesis that dogs affected with veterinary visit FAS treated with Aqua-AP at sedation acupoints (*An-shen*, GB-21) would demonstrate greater improvement in FAS clinical signs than untreated controls and would demonstrate noninferiority to dogs premedicated with the serotonin modulator, trazodone.

A variety of studies have demonstrated positive

results for treatment of anxiety, stress and fear with various AP techniques in a number of species. In one human clinical trial, AP was able to significantly reduce anxiety, stress, and depression ($p < 0.001$) in young adults and elderly subjects.²⁴ Improved sedation after AP has been demonstrated in several studies including elderly subjects with pneumonia on mechanical ventilation, patients undergoing cardiac surgery and critically ill patients in intensive care units.²⁵⁻²⁷ A systematic literature review of AP treatment for anxiety in humans concluded after review of a large body of studies, that real positive outcomes are consistently seen with statistically significant findings for AP's ability to mitigate stress and anxiety.¹⁰ Several rodent model studies in this literature review evaluated both behavioral and biochemical markers associated with mild chronic stress and demonstrated statistically significant improvement for the AP group versus controls.¹⁰

The literature is sparse for studies investigating AP versus pharmaceutical treatment for the relief of canine FAS. One clinical study using 24 healthy dogs divided into 2 groups in a cross-over design investigated mitigation of fear and anxiety with AP treatment during ear cleaning.²⁸ Each group of dogs was treated with DNAP or butorphanol injection before the veterinary procedure. There was detailed scoring of stress behaviors during the procedure. After a wash-out period, the dogs then received the opposite treatment. Regardless of group assignment, dogs that received DNAP at *An-shen* had significantly improved sedation ($p = 5.01 \times 10^{-8}$) when compared to the pharmaceutical treated dogs. Another study investigating canine stress during a clinic visit used 20 dogs divided into 2 groups (control and test) that were

treated with sham or real Aqua-AP at sedation acupoints (*An-shen*, GV-20).²⁰ The study showed behavior score improvement in both groups, however, the aqua-AP treated group had double the improvement of the control dogs. This study also measured saliva cortisol which exhibited significant data variation and was unrewarding in tracking stress responses of study dogs.²⁰

Similar to the previous study, the present study also lacked statistically significant changes in saliva cortisol concentration between T₀ to T₃₀ in all study groups. One plausible reason is that most study dogs underwent varying stress factors (e.g., vaccinations, venipuncture, nail trims) during the 30-minute period between saliva sample collections, potentially spiking the adrenal response and leading to spurious results. In addition, no distinguishing effects were considered in the study design that separated negative stress and positive stress (happy excitement), both resulting in saliva cortisol release. An unexpected finding in this study and interesting aspect of the group premedicated with trazodone for stress mitigation, is the apparent higher baseline saliva cortisol level and persistent FAS indicators in comparison to the other 3 groups. The study subjects in the premedicated group could be considered comparatively more stressed for the whole duration of their visit, despite the premedication with a serotonin modulating medication. A similar effect was reported in a study conducted in horses comparing stressed animals premedicated with acepromazine versus use of Aqua-AP at GV-1. The animals receiving the pharmaceutical demonstrated higher stress cortisol levels even though sedated when compared with the Aqua-AP group, which also demonstrated sedation.²⁹ Of interest in the present study is that both Aqua-AP groups' post-treatment cortisol levels were not higher than the premedicated group with a 0.05 significance level. From these findings it can be concluded that saliva cortisol measurement in this study provided limited insight into a patient's stress and FAS mitigation response to study treatments.

The conduct of this study had several challenges. Obtaining the required number of dogs to provide valid statistical analysis and that met study enrollment criteria was difficult to achieve in a timely fashion. This necessitated enrolling a subject population that did not have optimal uniformity. The over-representation of Golden Retrievers in this study may have skewed results as positive stress (happy excitement) was not distinguished from psychogenic stress.^{23,30} Other studies in university research settings limited their population variation by including only one breed (beagles, German shepherds), reducing potential result fluctuations due to body weight and breed differences.^{6,22,31,32} Age may have also been a factor. There was a statistically significant difference in mean age between the groups which likely created variations in animal responses. Older dogs may have habituation to activities at the veterinary clinic (good or bad) but could also be slower and less reactive than younger dogs. Dropout of dogs from the study (1 in each group) caused a mild lowering of statistical power for

group comparisons which probably didn't affect study results but must be considered in study limitations. Consideration is also given to a variable knowledge base in AP for veterinarians giving Aqua-AP treatments as not all participants were certified veterinary acupuncturists. This most likely had negligible effects as all associates were instructed on how to perform the simple Aqua-AP procedure at the 2 anatomic sites. Finally, the saliva cortisol testing with its inconsistent results in this study and others, shows limited application as a guiding component for stress evaluation in clinical settings due to unpredictability. More recent studies have explored the connection of stress and immunoglobulin A (IgA) levels as well as the neutrophil: lymphocyte ratio as a baseline for stress in dogs, in order to determine a more reliable and reproducible indicator for canine stress.^{22,32}

In summary, the findings of this study suggest that Aqua-AP at *An-shen* and GB-21 is an effective treatment for canine veterinary visit FAS. This technique produced statistically significant HR and FAS behavior reduction and was non-inferior to the pharmaceutical, trazodone. It is cost effective, uses easily accessible acupoints and can be administered after the animal arrives at the clinic versus pre-visit medications. Further research should be considered to advance understanding of stress mitigation options for FAS as well as development of more reliable ways to measure stress experienced by canine patients in the veterinary clinic.

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FOOTNOTES

- a. Trazadone, generic, multiple sources in USA
- b. Immunoassay Kit, cotton saliva collection rope, spin vial; Salimetrix, Carlsbad, CA USA
- c. Terumo syringes and needles, MWI Animal Health, Boise, Idaho, USA
- d. Vitamin B₁₂, MWI Animal Health, Boise, Idaho, USA
- e. Centrifuge, MWI Animal Health, Boise, Idaho, USA
- f. R version 3.5.2. The R Foundation for Statistical Computing, Vienna Austria; <http://www.R-project.org>

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